UNITED STATES DEPARTMENT OF AGRICULTURE

BUREAU OF ENTOMOLOGY

FOREST INSECT INVESTIGATIONS

PHYSIOLOGY OF TREES ATTACKED BY BARK BEETLES AND THEIR ASSOCIATED FUNGI

by

R. W. CAIRD
Field Assistant

Asheville, N. C.
1931

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The writer wishes to thank all who helped in this study. Mr. R. A. St. George and Dr. H. M. Nelson were especially kind in offering suggestions. The scope of the work was greatly increased through permission to use the equipment of the Forest Pathology Laboratory of the Bureau of Plant Industry.

The following field assistants helped collect data at various times during the summer:

Bernard J. Huckenpahler	University of Minnesota
Lyall E. Peterson	" " "
Noel D. Bygant	Purdue University

I am confident that their work, presented in this report, will "stand up". Mr. Huckenpahler contributed considerable skill in chipping and the method of "stepping" trees into dye solutions. Mr. Bygant worked with the fungus cultures, and invented a system of keeping records on the cultures which brought order to the chaos of my system. Mr. Peterson also worked with the fungus cultures, as well as in the field.

GENERAL SUMMARY AND CONCLUSIONS

The study was aimed to answer two main questions:

1. What kills the tree? (agency)
2. How does it do it?

The report fails to answer either question.

1. <u>What kills the tree?</u> (agency)

The choice here is between two agencies, the barkbeetles and the bluestain fungi. The report stresses the importance of the mechanical injury due to the tunneling of the phloem by the beetles, in bringing about the diseased condition. The inter-dependence of the two possible agencies which might cause the death of the tree is brought out by the failure of the fungi to grow when the entrances to the galleries were blocked.

The effect of the fungus working alone is shown by the inoculation work. The fungus is able to kill the tree when acting alone. But it is entirely probable that the wounding of the trunk and opening of the bark takes the place of the beetle galleries.

The effect of the mechanical injury of gallerying is difficult to show. Close examination of trees dying from the disease indicates that over large areas, simple drying is taking place in the absence of the fungus. Also, in a single inoculated tree it is possible that the fungus failed to penetrate the wood, but drying occurred and the tree was being killed. In this case, it is possible that simple drying

from the wound was taking place.

It is suggested that possibly the simple drying from the openings of the galleries, and drying speeded up by the penetration of the fungus, takes place in the tree dying from the disease.

2. How does the agency kill the tree?

Here there are a number of possible ways in which the agency may be working. No satisfactory explanation can be reached until the most probable mechanisms are experimentally tested, and the results shown to apply to the diseased tree.

The report deals extensively with the accumulation of air in the tracheids in consequence of drying as a mechanism for the stoppage of conduction. An attempt is made to discover the mechanism of the stoppage of conduction in inoculated trees, in the hope that a clew as to the mechanism in the diseased tree may be found. It is apparent that the death of the beetle-attacked tree, and the death of an inoculated tree, are similar as far as drying and the stoppage of conduction are concerned. However, no causal connection is demonstrated between the accumulation of air in the tracheids and actual stoppage of conduction. This uncertainty will continue until it is shown experimentally that no other mechanism fits the conditions.

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PHYSIOLOGY OF TREES ATTACKED BY BARKBEETLES AND THEIR ASSOCIATED FUNGI.

PART III

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PART I

METHODS USED IN TREE ANALYSIS

PART II

PHYSIOLOGY OF TREES ATTACKED BY BARKBEETLES AND THEIR ASSOCIATED FUNGI.

During the 1929 and 1930 field season a study was made of the control of barkbeetles in pines by means of poison solutions into the sap-streams of the infested trees. It was thought that a fuller knowledge of the disease would be helpful in applying this method of control. The work described below is also intended to help in devising better methods of control for this disease.

The physiological study is a part of the larger program concerning the general biology and control of the southern pine beetle, Dendroctonus frontalis Zimm., begun in 1925 at Asheville, N. C., on the Bent Creek Experimental Forest. These studies are carried on in cooperation with the Appalachian Forest Experiment Station of the U. S. Forest Service. The 1931 season marked the beginning of an intensive study of the abnormal physiology of this barkbeetle-fungus disease. This progress report is limited to the writer's work, and presents the present status of the problem rather fully, in order to serve as a basis for restating the problem and planning the next season's work.

1. THE PROBLEM

The methods of attack and collection of data are designed simply to answer two main questions:

I. What agency kills the trees?

II. How does it do it?

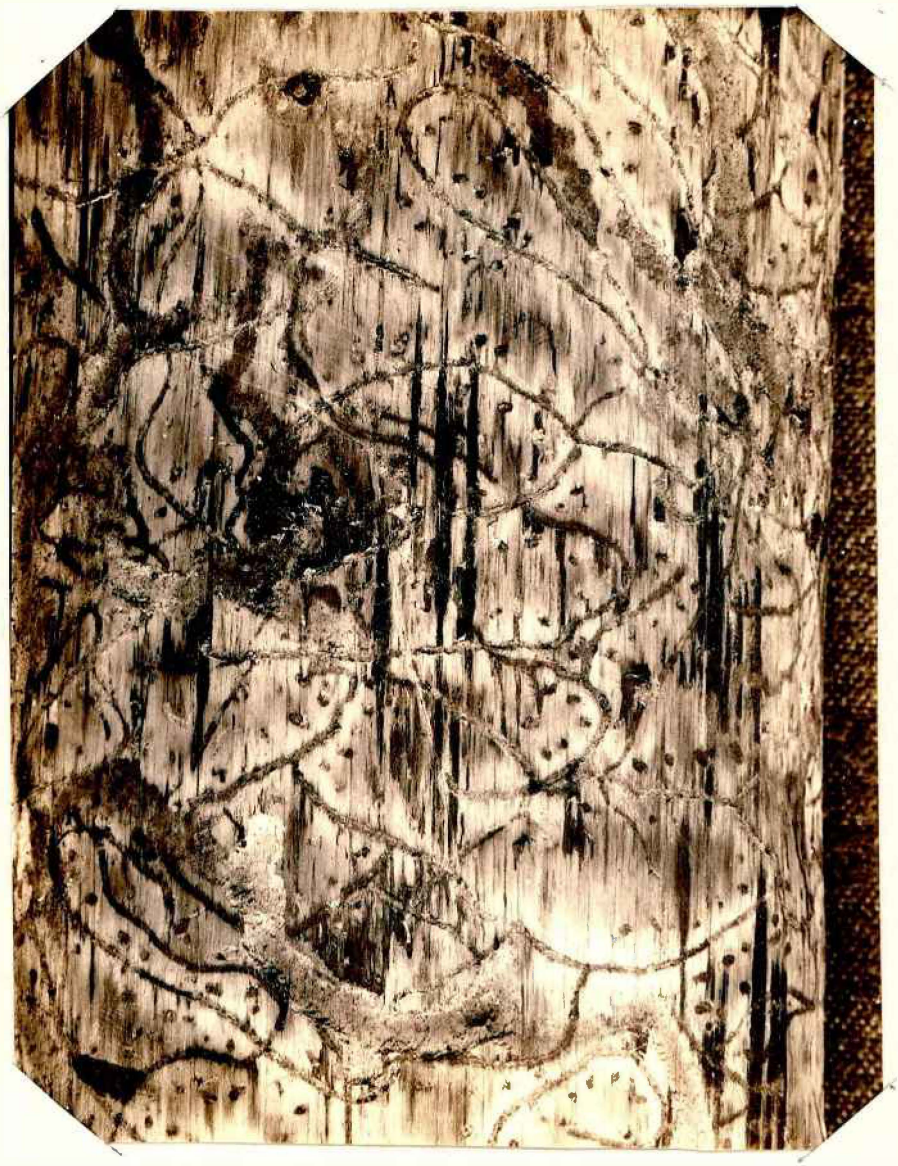
2. DESCRIPTION OF THE DISEASE

The project described in this report was limited to a stand of shortleaf pine (Pinus echinata Mill.) about 30 years old. The diameter at breast height of the trees used was about 5 inches, and the height about thirty feet. A few pitch pines (P. rigida Mill.) were taken for preliminary work from a different stand.

Attacks of the southern pine beetle were induced by caging bark containing beetle on the trunks of healthy trees. (Plate III, page 7) When the beetles attack the caged trees other beetles in the vicinity are attracted and enter the neighboring healthy trees. This study is limited to trees attacked by the southern pine beetle acting as a primary beetle; that is, the trees were healthy up until the time they were attacked. Secondary beetles are attracted to the trees attacked by the southern pine beetle within two or three days.

An attempt was made to determine the actual conditions in the trees at various stages of the disease, in order to secure clews to the answers of the questions stated above. Moisture data were collected at intervals up the stems of

PLATE I



(St. George, 1927)

BEETLE GALLERIES AND BLUESTAIN

This represents a late stage, as witnessed by the pine sawyer (Monochamus sp.) gallery. (Large gallery near bottom of picture) The southern pine beetle galleries are conspicuous, and have the pupal chambers showing. This is a picture of the surface of the wood, and shows the bluestain extending in vertical streaks from the galleries. The bluestain shows fruiting bodies.

trees at three or four foot intervals. (Plate II, fig. 1, page 5). For the purpose of presentation, the different tissues will be given as follows:

a. Drying of the phloem (inner bark)
b. Drying of the leaves
c. Drying of the wood

a. Drying of the phloem (inner bark)

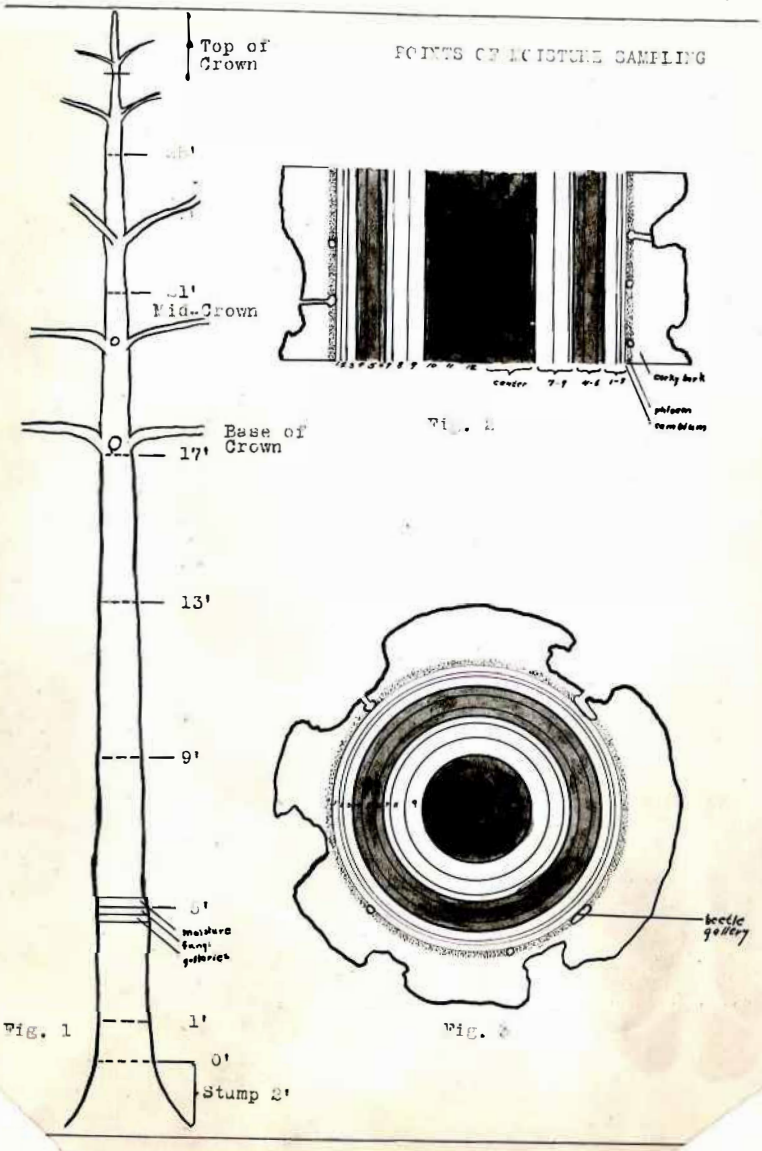
The beetles tunnel through the protecting corky layer of bark to the phloem, and gallery this quite extensively. The eggs are laid in the phloem along the galleries, the new brood developing in the phloem region and later in the outer bark. The conditions necessary for brood development are apparently quite exacting and complex. This study is limited to the mechanical injury caused by the tunneling of the phloem, and to the fungi which the beetles probably introduce.

The phloem quickly turns brown along the galleries, until all of the phloem between the galleries is brown. Bluestain (Ceratostomella sp.) can be observed darkening the phloem within a few days after the tree is attacked. The phloem probably serves as a substratum for the rapid growth and spread of the fungi associated with the beetles, and probably serves as food for the beetles. The somewhat detailed description of how the phloem dries out which follows is given because it illustrates the close connection between the opening of the trunk by the beetles and drying, and

PLATE II

POINTS OF MOISTURE SAMPLING

PLATE II 5.
POINTS OF MOISTURE SAMPLING



because we wish to let this statement be a summary of the moisture conditions in the phloem, with a view toward droping the moisture determinations of the phloem from future work.

In the summer's work, the course of the disease was followed by selecting trees at various numbers of days after the beetles had attacked them and inoculated them with fungi. If conditions were the same in all trees, we would expect these trees to show successive stages of the disease. This is the case in general, but it was found that some trees die more quickly than others, because of the more rapid development of the disease in particular trees. In order to reduce the variation between trees due to the quicker development of the disease in some trees, in some cases the trees have been arranged according to the development of the disease asindicated by the successive loss of conduction in the outer rings. In such cases, the loss of conduction is the measure of the stage of the disease, rather than using the number of days which have elapsed since the tree was attacked and inoculated as a measure.

There is a sudden drying of the phloem along the beetle galleries within a few days after attack. This is illustrated by Graph I, page 8. The loss of moisture is most rapid in the region of the stem first attacked, from about 3 to 12 feet. The southern pine beetle enters first at about this height, and is soon followed by the secondary barkbeetle Ips avulsus Eichh. and I. grandicollis Eichh. I. avulsus works in the tops of the attacked trees; in the trees [illegible]

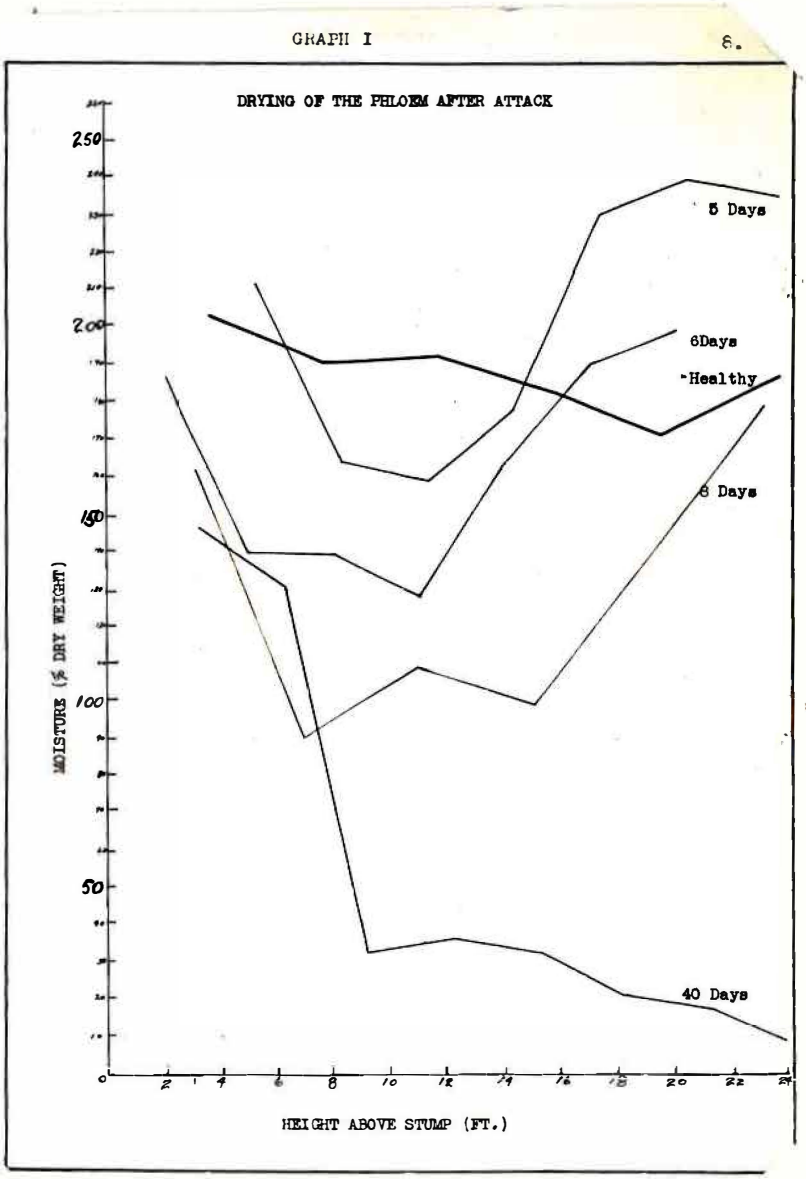
PLATE III.

INDUCING BEETLE ATTACKS

The method used to induce attack of the southern pine beetle (Dendroctonus frontalis) on shortleaf pine by caging sections of bark containing maturing broods of the beetle. The white surrounding trees indicate pines infested as the result of attraction of the beetles to the area because of those emerging in the cage and attacking the tree.

GRAPH I

DRYING OF THE PHLOEM AFTER ATTACK



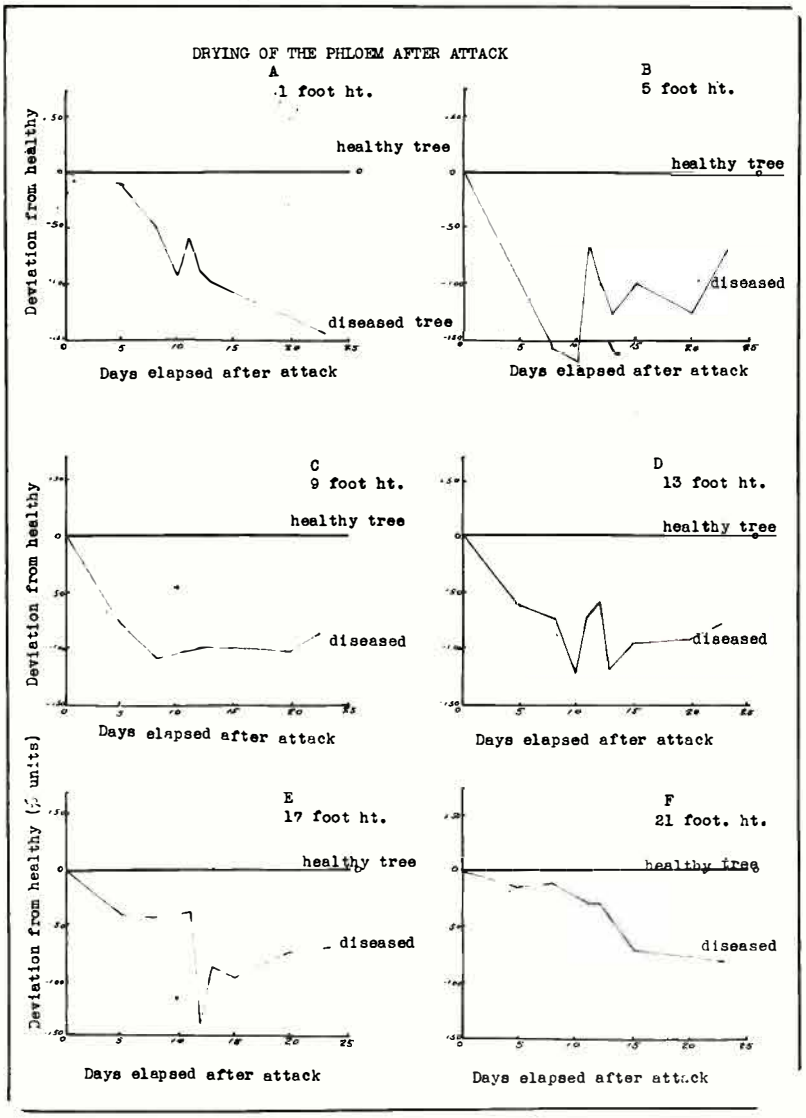
concerned in this study, it galleries the phloem from about 17 feet to the leader. Thus the bark for the entire length of the stem is punctured by the beetles. I. grandicollis works in the same region at about the 15 foot level.

The region of drying indicated by the dip in the line of Graph I representing the fifth day after attack increases as the beetles "fill in" along the trunk. The disease develops from the point of first attack and spreads upward and downward. The phloem in the top of the tree gradually loses its moisture until it becomes as dry or dryer than that at the middle of the tree. The final position is probably represented by the graph line for 40 days. In all cases shown, the base of the tree retains its moisture content longer than the upper part of the trunk. Eventually, of course, the base dries out. The cause for the higher moisture content of the phloem at the base is probably the lack of beetle attack in this region, and perhaps also the failure of the bluestain fungi to grow at this level.

At any given point on the trunk, the moisture is gradually lost from the phloem, and different heights lose their moisture at different rates. This is shown in Graph I, but better proof is presented in Graph II, page 10. Here the selection of data is avoided. Although this graph represents a series of trees rather than a single tree, it is

GRAPH II

DRYING OF THE PHLOEM AFTER ATTACK



believed that it approximates the course of the disease in a single tree. The 5 foot level dries out and the phloem tissue is killed there more rapidly than at the other levels. The phloem at this height is quite dry after only five days. The extremities of the trunk do not show marked divergences from the healthy tree until considerably after the phloem at mid-trunk is dry and dead.

b. Drying of the leaves

Purpose: If the leaves were injured in some way by the action of some factor in the trunk, they might be unable to draw water through the stem, and thus the loss of conduction by the wood of the trunk would be accounted for. At any rate, more information was needed concerning the leaves, since we consider them to assist in the drying of the trunk.

We wished, specifically, to determine which part of the crown showed the first effects of the disease, and also which year's needles (1929, 1930, 1931) showed permanent drying first.

Method: Some easy method of securing a measure of the health of the leaves was necessary, and moisture determinations were chosen as the easiest.

The moisture determination of the leaves was made a part of the routine of tree analysis adopted for the 1931

season. In order to minimize the effects of diurnal, and other changes due to weather conditions, almost all trees analysed in 1931 were cut between 6:15 and 8:30 A.M., and for each diseased tree analysed, a similar healthy tree was also analysed. The leaves were left attached to the branches after the trees had been felled, and about two hours elapsed between the time the branches were cut and the plucking of the leaves in the laboratory. The error involved in this delay was not determined.

The leaf samples were of a size determined by the number which could be conveniently packed into a one pound size paper bag. The weight of the samples generally approached 45 grams green weight, although in some cases only about 5 grams of leaves were available. The needle sheaths generally remained attached to the 1930 needles, but the 1931 needles came free without the sheaths.

The following division was used:

Base of crown	In each case, 1929 needles
Mid crown	not taken, 1930 and 1931 needles
Top of crown	taken.

The "base of the crown" is formed by the first branches bearing living needles, about 17 feet from the ground (Plate II, fig. 1, page 5) The "mid-crown is made up of the branches half-way from the lowest living branch to the leader. The "top of the crown" consists of the leader, and a few side branches just below it.

Results: Healthy leaves formed in 1931 show a higher moisture per cent. than the older 1930 and 1929 needles. (Graph III, page 15) These same leaves

No. Page 13

no page 13

have approximately the same moisture per cent. regardless of whether they are taken from the base of the crown, mid-crown, or top of crown. The 1930 needles also show this uniformity in moisture content throughout the top of the tree.

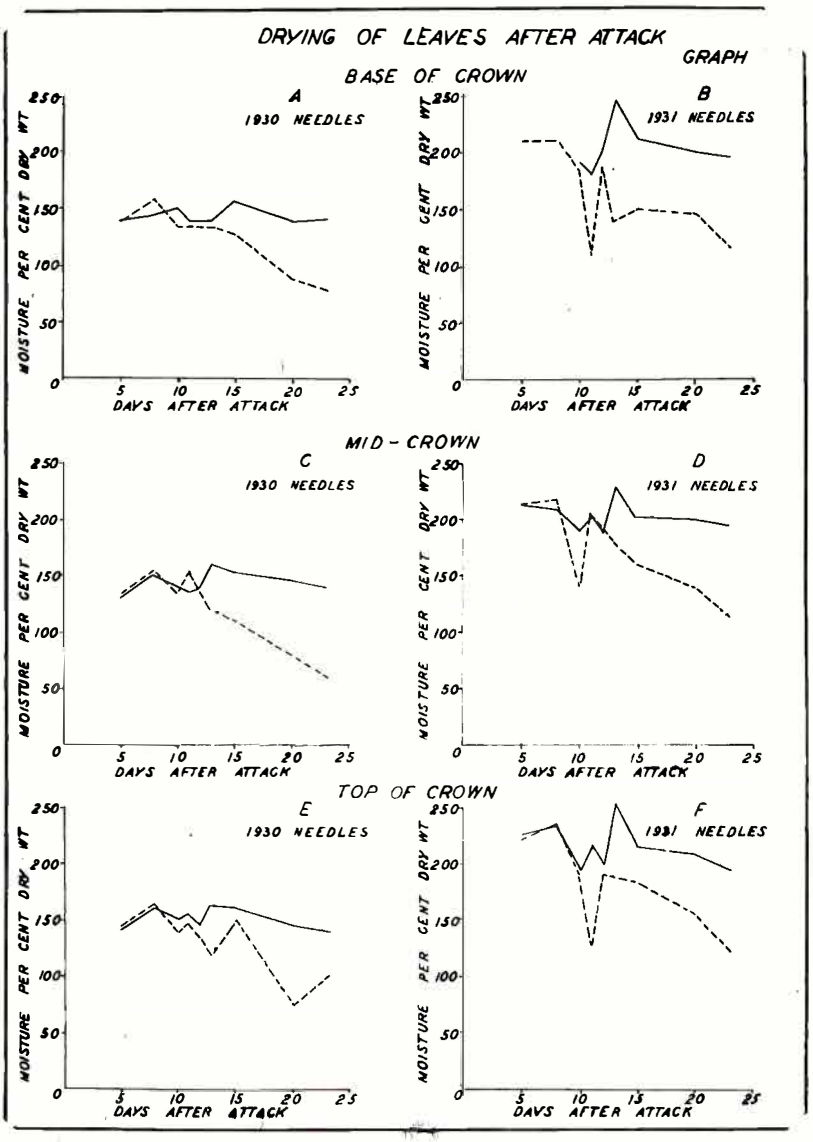
The leaves on diseased trees do not show any marked divergence from the healthy until about 13 days after attack. There is a gradual drying out after this point, and apparently the whole crown dries out at about the same rate. The leaves formed in 1930 and 1931 seem to dry out at the same rate regardless of whether they are located in the base, mid- or top of the crown.

The critical point at which there is a marked divergence of the diseased leaves from the healthy is at 13 days after attack. If the permanent loss of moisture by the leaves is significant as a measure of the health of the leaves, this would mean that the leaves are not affected until about ten days after the trunk has been injured.

At the time the leaves show a significant divergence from the healthy, the foliage of the trees had not shown any noticeable yellowing. At thirteen days, and at 15 days, the leaves were still green. At twenty days, the 1929 needles were turning yellow, the 1930 were fading slightly, and the 1931 needles were still green. After 23 days, the 1929 needles were light brown, the 1930 turning yellow, and the 1931 needles were still green. Apparently, the color change does not occur until after the leaves have shown permanent loss of moisture.

GRAPH III

DRYING OF LEAVES AFTER ATTACK



By the time the leaves showed incipient drying (13 days), the first three rings of the trunk from 5 to 13 feet above the stump had been non-conducting for 8 to 10 days. The fourth to sixth rings had been non-conducting for the same distance for about 5 days. The seventh to ninth rings were still capable of conducting, as well as the center.

Discussion: The significance of these data seem to be that the leaves are killed due to a disturbance in the conduction in the trunk, and that the conduction in the trunk stops due to other causes than injury to the leaves. However, no evidence is presented to show that the loss of moisture is a good measure of the health of the leaves in the early stages of the attack.

Conclusions:

1. The diseased condition in the trunk affects the moisture content of the leaves after about thirteen days.

2. Attention should be directed toward the conditions in the trunk, rather than the conditions in the leaves.

3. The 1930 needles lose their moisture at the same rate, regardless of their position in the crown. The same is true of the 1931 needles.

c. Drying of the wood

This is almost certainly a vascular disease in which the death of the top of the tree follows the loss of the capacity of conduction by the stem. That this is the case in beetle-attacked trees has been suspected for a number of years. The first proof of the loss of conduction in beetle-attacked trees was found in the experiments carried on in 1929, in which trees stepped in dye solutions failed to take the solution up the outer rings of the wood.

The drying of the wood, following the beetle attack and the inoculation of the tree with fungi (by the beetle), was advanced as an explanation for the stoppage of conduction of water by the stem from the roots to the leaves. This explanation was based in part on moisture sampling done in 1930.

Method: The method of collecting wood for moisture determinations is described on page 83. To this it may be added that the trees used were stepped in dye solutions, in order to determine which layers were conducting. The effect of the introduction of the dye solution is to raise the moisture per cent. in the rings which are conducting.

Results: In general, the tree trunk dries out first where the beetles first open the bark, as described for the phloem, from about 3-12 feet. Drying progresses

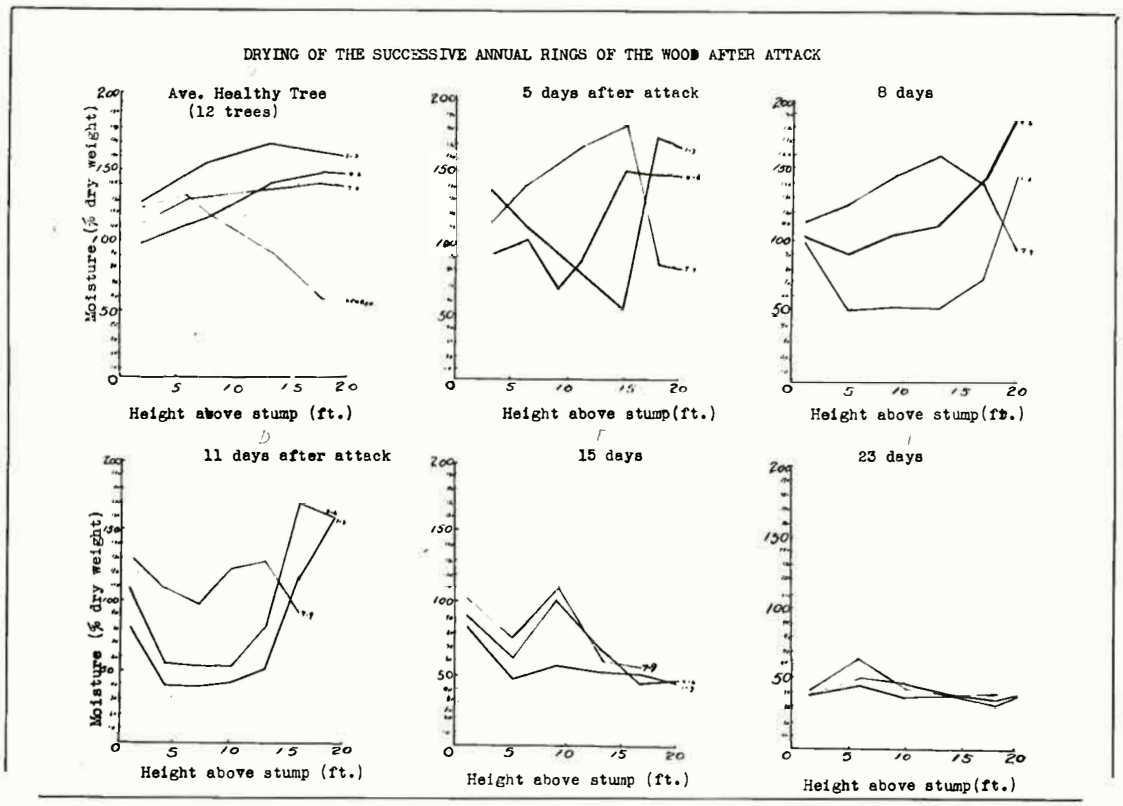
upward and downward from this region as the beetles "fill in" and the phloem is injured and the tree inoculated with bluestain and other fungi. (Graph IV, page 19) Drying proceeds towards the center of the tree first in the region of first beetle attack, with a lag occurring progressively upward and downward, probably correlated with the difference in the time of attack of these portions by the beetles.

The chief difficulty in understanding this disease lies in the fact that there is a coincidence of all the possible agencies and conditions which they set up at the point of first attack, and their development is greatest here. This is the region of first beetle attack, first fungus development first drying, and other developments. This has lead to confusion. The aim is to find out what the conditions are and then to try to determine their effects when acting alone.

The above description is based upon the data collected. Sample data are presented in Graph IV, page 19. Instead of showing the usual moisture gradient of the healthy tree (A) the lines break to give a dip where drying has occurred. The first loss of moisture occurs in the 1-3 rings, although this stage was not secured in the 1931 work. The drying progresses inward to the 4-6 rings (B and C) with the remainder of the rings to the center showing no apparent drying. The zone of drying extends toward the top and base of the tree. The 7-9 rings show a break next, and finally the center. The

GRAPH IV

DRYING OF THE SUCCESSIVE ANNUAL RINGS OF THE WOOD AFTER ATTACK



line representing the center is omitted in all of the graphs of Graph IV except A in order not to confuse the picture. Graph A of this series shows the typical drop in the moisture content of the center of the tree as the top is approached. This drop is commonly encountered in the other ring groups as they in turn represent the center of the tree near the top of the tree.

The top of the trunk shows the effects considerably after the center of the trunk, and the base of the tree dries out very much more slowly. The final stages, (E and F) represent conditions after 15 and 23 days. The top of the tree gradually loses its moisture, but the base still has a considerable amount. The various layers have nearly the same moisture content. The stage represented by (F) still has considerable moisture in the base, although it is not shown in the graph. The tree was cut above the moist zone in the base.

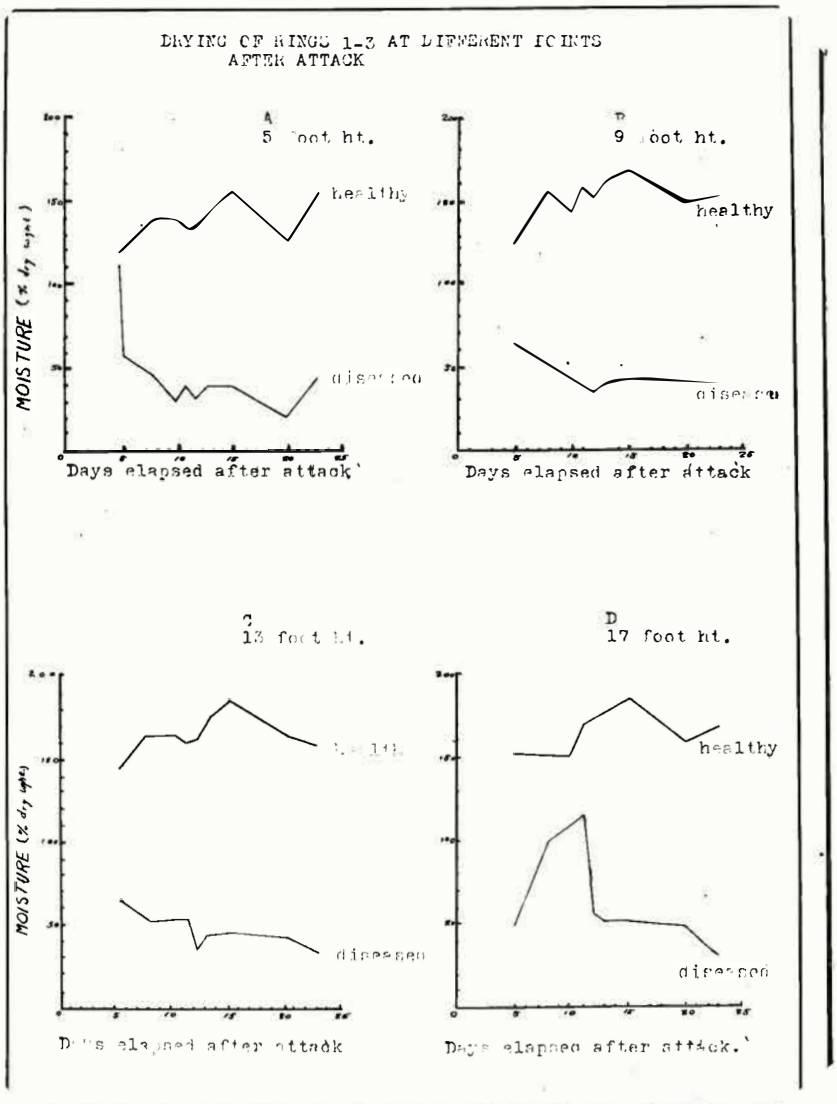
Graph IV represents an illustration of the course of drying, since the individual graphs were selected.

d. Drying of the wood and loss of conduction

If it could be shown that there was no correlation between the drying of the conducting layers and the loss of the ability to conduct, we would feel justified in seeking some other mechanism to account for the stoppage of conduction. On the other hand, if a correlation were found to exist, as is the case, there is no assurance that the

DRYING OF RINGS 1-3 AT DIFFERENT POINTS AFTER ATTACK

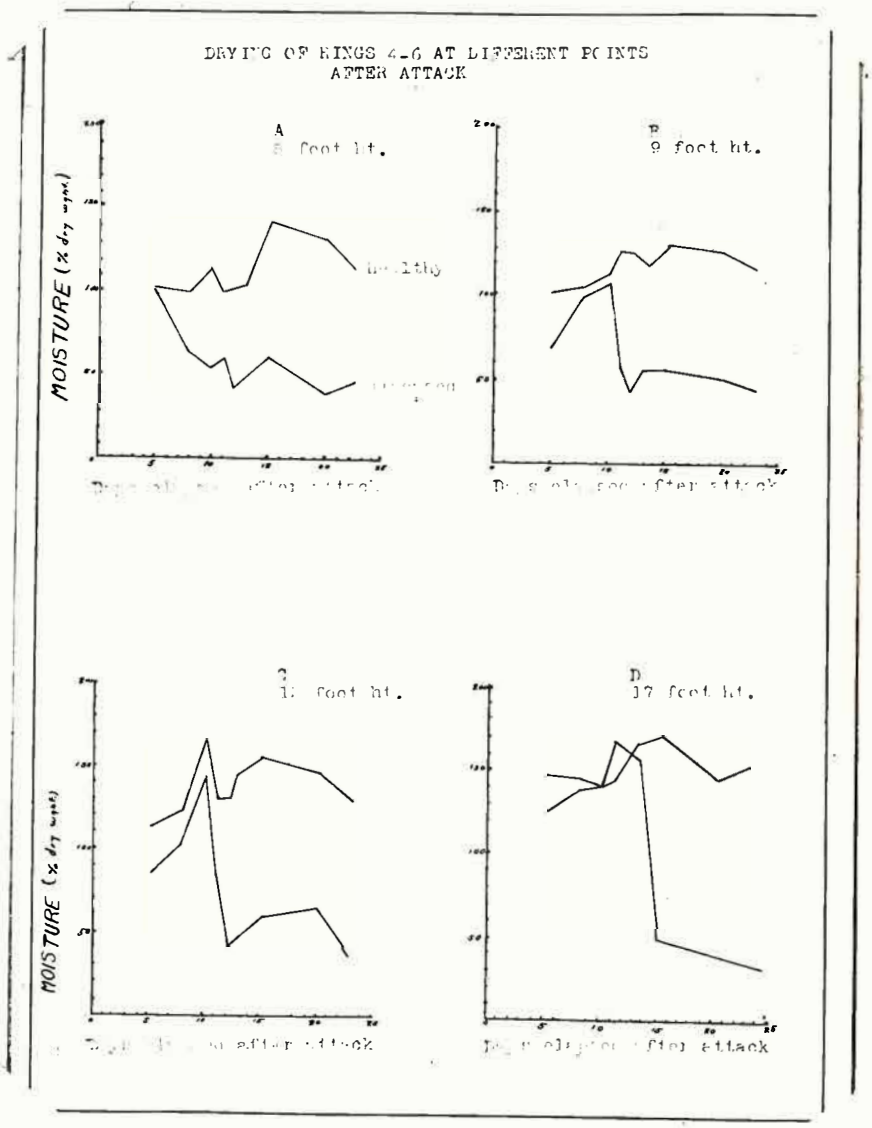
GRAPH V



GRAPH VI.

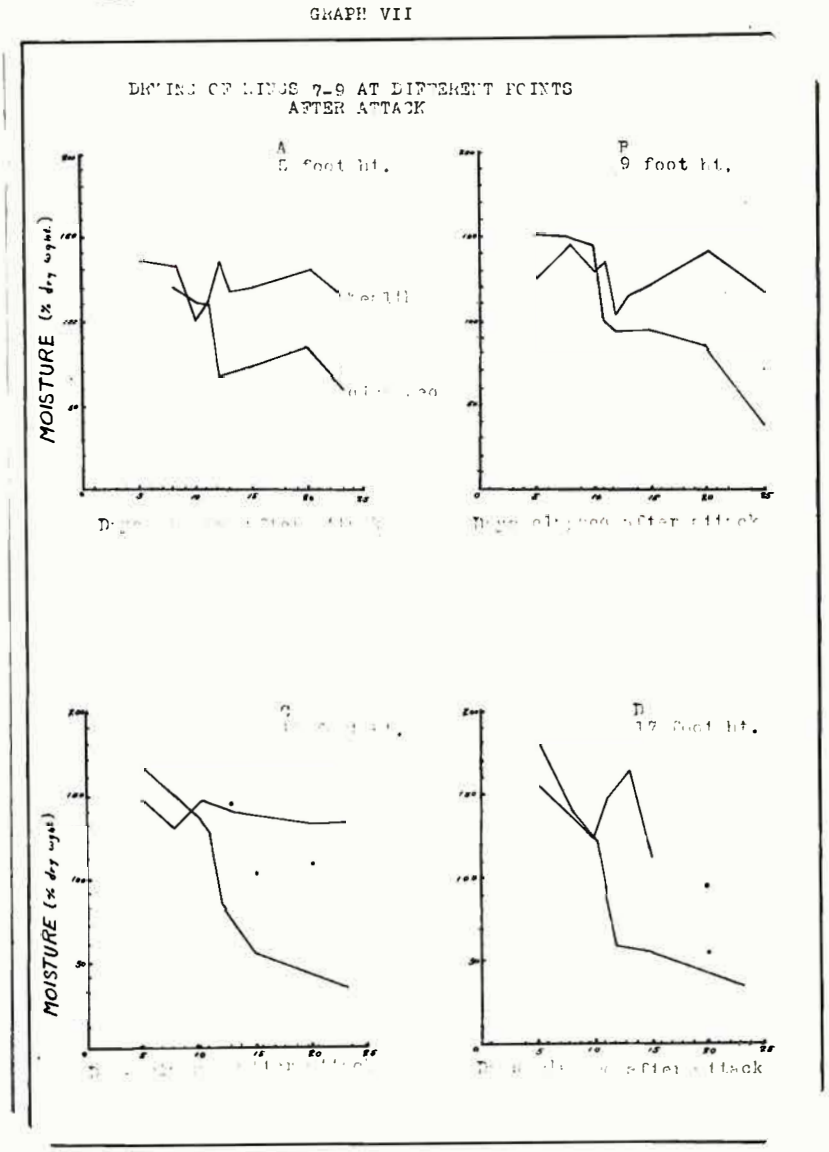
DRYING OF RINGS 4-6 AT DIFFERENT POINTS AFTER ATTACK

GRAPH VI



GRAPH VII

DRYING OF RINGS 7-9 AT DIFFERENT POINTS AFTER ATTACK



two events are causally connected. The suggestion is that the accumulation of air in the tracheids as the wood dries would offer sufficient resistance to the passage of water through the tracheids to stop conduction.

In almost every case, it is found that the annual rings which have dried out subsequent to the attack by the beetle and the inoculation of the trunk are also non-conducting. Graphs V,VI & VII, p. 21, have been prepared to show this relationship. The graphs have been limited to 5,9,13, and 17 feet above the stump, since these probably represent the zone of most importance. In Graph V, A,B,C, and D, rings 1-3 have dried out and lost the ability to conduct within eight days after attack, at the 5, 9, and 13 foot levels. The variation in the 17 foot level represented by (D) is probably due to the variation in the rate with which the disease develops in different trees. Experience has shown that rings 1-3 dry out later and conduct longer at 14' and 17' than the same rings lower on the tree.

The succeeding ring groups, 4-6 and 7-9, diverge from the healthy and lose their ability to conduct at progressively longer periods after attack and inoculation by the beetle. Graph VI, A, representing the 5 foot level, diverges from the healthy between 5 and 8 days after attack, but the 9 and 13 foot levels do not lose their moisture until the tenth day after attack. The 17 foot level does not dry out until about 15 days have elapsed. These rings also appear to lose their ability to conduct.

Further investigation will doubtless improve these graphs. They must, of course, be intrepreted with reference to the healthy tree. Thus, in Graph VII, (D), representing rings 7-9 at the 17 foot level, there is commonly a sharp drop in the moisture content of the healthy rings as they become the center rings near the top of the tree, and they commonly do not conduct solutions.

e. The accumulation of air in the tracheids, and the loss of the ability to conduct solutions.

The idea was advanced that the loss of moisture and the accumulation of air in the trunk of the tree offers a mechanism for the stoppage of conduction. In order to determine more closely the actual amount of air which accumulates in the tracheids, an indirect measurement of the air content of the groups of rings was attempted.

The computations rest upon the direct measurement of:

1. the green weight of the sample
2. the dry weight of the sample
3. the volume of the sample

Measurements 1 and 2 need no explanation. Measurement 3 is described on page 24. The volume of air in the sample was computed as the per cent. its value represented of the total volume of the block of wood, rather than the per cent. of the total volume of the lumini of the wood cells occupied by air. The following formula was used in determin-

ing the per cent. of air:

$$\frac{\text{Vol. Block} - (\text{vol. wood substance} + \text{vol. } H_2O) \times 100}{\text{Volume of Block}} = \% \text{ Air}$$

The volume occupied by wood substance (the actual volume of the wood in the cell walls, in contrast to the volume occupied by the cell walls and the cellular cavities) was determined according to the following excerpt from a letter to us from Mr. H. W. Bateman, of the Forest Products Laboratory:

"The dry weight of the wood in grams divided by 1.54 --the specific gravity of wood substance--gives the equivalent weight in grams of an equivalent volume of water, and since a gram of water is equivalent to a cubic centimeter, this equivalent weight can be taken as the volume in cubic centimeters occupied by wood substance."

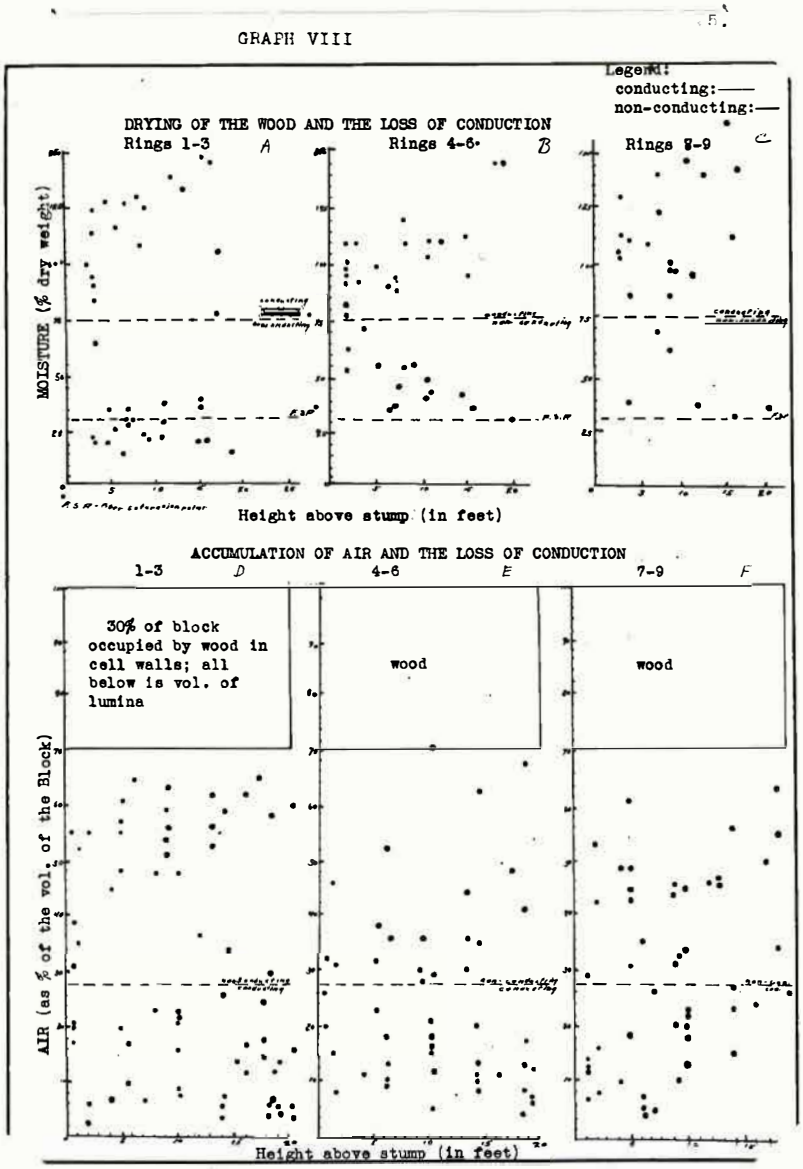
We are responsible for any errors which may arise from the use of the formula for determining the air content. The computed air volume varies inversely as the moisture content, since it is determined largely from the moisture data. There is considerable variation in the air determinations, probably due to errors in measuring the volumes of the wood. The data are valuable since they present a clearer picture of the actual conditions within the cells.

The chief interest, of course, lies in the air content of conducting as opposed to non-conducting wood. Conducting

GRAPH VIII

DRYING OF THE WOOD AND THE LOSS OF CONDUCTION

ACCUMULATION OF AIR AND THE LOSS OF CONDUCTION



layers have a low air content, while non-conducting layers have a high air content. This is shown by Graph VIII, page 25, D. E. and F, representing the conducting and non-conducting tissues for all available trees. The moisture data, based on dry weight, are given on the same graph in A, B, and C. In all cases, a red dot indicates non-conducting tissue, and a black dot, conducting.

Regardless of whether conduction and air content are causally related in these graphs, there must be a line of demarcation between the conducting layers with the high moisture content, and the non-conducting layers with low moisture content. In graph A, rings 1-3 represented by the red dots have probably dried out far below this critical line. Rings 4-6, (B), are closer to this hypothetical line dividing the conducting from the non-conducting. Rings 7-9,(C), show a still closer grouping to the "line". The same argument may be given for the graphs representing air content (D, E, and F). On the whole, there is a fair separation of the conducting and non-conducting values, with an intermingling along a line at which they may be assumed to be on the borderline between conducting and conducting.

If we assume that the fiber saturation point for this wood is 30% of the dry weight (which is probably 2 or 3 per cent. units high), and if we assume further that any per cent. higher than 30 represents free water, then Graph VIII, A. B, and C show the relative conditions in these layers as

regards free water in the cell cavities of the tracheids. There is little or no free water in the non-conducting rings in A, representing rings 1-3. There is still some free water in rings 4-6(B), and still more in rings 7-9. Evidently, stoppage of conduction has occurred while there was still free water in the lumina.

Air volume determinations presumably tell how much free water and how much air are in the lumina. The average per cent. of the volume of the block of wood occupied by wood substance was 30% for several trees for which it was figured. Considering the critical line to fall between 25 and 30% of the volume of the block occupied by air, this would be about 40% of the volume of the lumina. Then we would have the air occupying 40% of the lumina and the free water 60%. This, of course, is a gross approximation which will be changed as more work is done.

f. Penetration of the wood by fungi, and their relation to conduction.

Purpose:

The beetles puncture the bark, gallery the phloem, and inoculate the phloem and surface of the wood with fungi. The tree dries out rapidly at the mid-trunk, the fungi make rapid growth and penetrate to the center of the tree, the trunk becomes non-conducting, and the top of the tree dies. On purely theoretical grounds, there is reason to believe that the tunneling of the bark and the attendant drying of the wood is sufficient to account

for the rapid death of the tree. On the other hand, there is equal reason to believe that the penetration of the fungi and their attendant action on the wood, such as causing it to dry out rapidly, would account equally well for the rapid death of the tree. Personally, I am prejudiced in favor of an explanation which pictures a combination of the factors described in the two explanations given above. The following description attempts to present the relation between the position of the fungi in the trunk, and the stoppage of conduction, at different periods of the attack.

If it could be shown that a certain fungus, as the bluestain fungus,(Ceratostomella pini Wmach), was always in close proximity to or in the region in which stoppage of conduction was taking place, we would be a long way toward establishing a causal relationship between it and the conditions stopping conduction. On the other hand, if it can be shown that such a fungus is not always present at the seat of the disturbance, but that it tends to follow in after the disturbance, then the causal relationship would not be so clear. It was with these ideas in mind that we undertook the fungus culture work.

Methods: The fungus culture work was made a part of the routine of tree analysis. The sampling points were chosen at the same points as the moisture determination and air content work, in order to have these data to correlate with the fungus isolations. A separate cross section was cut off about 1 1/2 to 2 inches thick, and the surface mapped for bluestain and dye solution. This same section was then marked for isolations.

The only culture medium used was "Bacto Malt Agar", as sold by the Digestive Ferments Co., of Detroit, Mich. The pH was not adjusted, and hence was about 5.5.

It was frequently found that planting from the phloem and first ring gave mixtures of fungi. In order to separate them, advantage was taken of the difference in growth rates; transfers were made from the edges of the growths. Bacteria and yeast often occurred in pure culture, or in isolated colonies. When mixed with the higher forms, the yeasts and bacteria were isolated in pure culture by streaking them on plates of agar with the point of a dissecting needle, and making transfers from the plate. When Trichoderma spp. or Penicillium spp. occurred in pure culture a note was taken of them and the culture destroyed. If in mixture, transfers of the desired fungi were secured and the plate destroyed.

In addition to the precautions observed above, to keep the "weed" fungi out of the laboratory, care was taken to open no Penicillium or Trichoderma cultures in the inoculation chamber. The usual precautions of washing the hands and fore-arms with alcohol before working in the inoculation chamber, wiping down the table with alcohol before placing cultures on it, keeping the Petri dishes in museum jars while the fungi were growing, were observed. In addition, strips of adhesive tape were used on Petri dish cultures to avoid accidental opening. Original cultures were kept until it was sure that the fungi were growing in test-tubes, and generally in pure culture. An attempt was made to secure every distinct fungus from each sampling point in pure culture, to be held until a final examination was made.

The method of noting the fungi was to give each new form which appeared in the cultures a number, and this culture was held as a type fungus with which to compare all subsequent cultures. When the same fungus reappeared its presence was noted by the type number, and the culture destroyed. Thus at the end of the work for the season we have on hand the type cultures, and a record of their occurrence in the trees examined.

Dependence was placed of a simple comparison of the microscopic culture tubes of the pure cultures of the fungi, without examination. The number of forms encountered was limited (perhaps 20, excluding possible duplicates), and where doubt as to identity arose due to slightly different growth forms, the culture was made a type fungus and saved for more careful examination. There was no difficulty in distinguishing bluestain as far as the genus, and in most cases other fungi were easily "spotted."

It was found that frequently in the early stages of attack, there was no apparent bluestain, but that conduction was stopped for several rings inward. The system of taking cultures only from bluestain wedges was dropped entirely and a system adopted which would trace a single pencil of water from the base of the tree to the top. To do this, the cross section was oriented as shown in figure1, Plate II, page 5, so that a line could be traced up the stem. Samples were taken at three or four foot intervals, so that this work must be considered as an attempt at securing averages of the conditions which a stream of sap would encounter on its way from the roots to the leaves. An attempt was made to follow four lines up the trunk, as shown in figure 3 of Plate IV, page 31, by the letters A. B, C, and D. The portions along the lettered radii bounded by the broken lines were cut out by striking downward on the back of a hunting knife held along the lines.

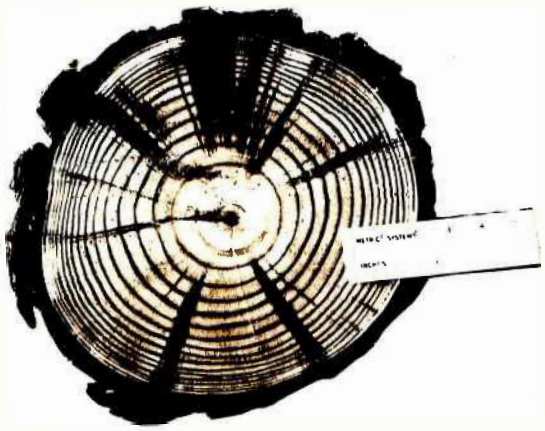
The block from which cultures were to be made was then lettered, and the rings from which plantings of the tissues were desired were marked with a pencil. This was to avoid errors in taking the tissue. The entire block, containing the trimmed corky bark, the phloem, and the wood from the cambium to the center of the cross section, was dipped in 85-95% alcohol, ignited, and the alcohol permitted to burn until almost out before extinguishing it. The corky bark was left on the block to protect the phloem from the effects of heating.

After flaming, the block was transferred to the inoculation chamber, which was kept relatively free from spores by frequent washing of the inside with alcohol. A sharp scalpel was used to secure the tissue from the block. After heating the blade of the scalpel in an alcohol flame, the scalpel was transferred to the inoculation chamber and the blade placed along the springwood, and struck so as to split the wood downward between the successive summerwood rings. An attempt was made to have the scalpel start the split in the wood, so that the pieces could be pried apart with the fingers, without of course touching the surfaces from which the tissue was to be taken. This was done to avoid dragging chance spores down with the knife.

The tissue for planting was secured by simply taking the scalpel and cutting a good-sized piece of wood from the surface exposed by splitting. This chip was then transferred to the Petri dish or test tube on the tip of the scalpel, or dropped in directly from the block. Whenever Petri dishes were available they were used for plantings. Three or four chips were taken from each ring samples, in order to increase the chances of securing the fungi. When it was necessary to use test tube slants, only one chip was taken. Where a mixture of fungi was expected, as in the phloem and first ring, Petri dishes were used for planting.

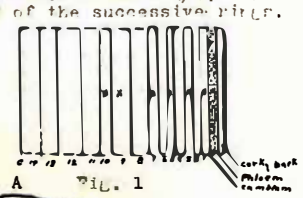
PLATE IV

PLATE IV 31.



Cross section [illegible] stage in the [illegible]. Blue stain well up to center. Reduced 1/4. Note the variation in the width of the successive rings.

Right: A section of cross section below prepared for fungus sampling. Block from B below to center.



A Fig. 1

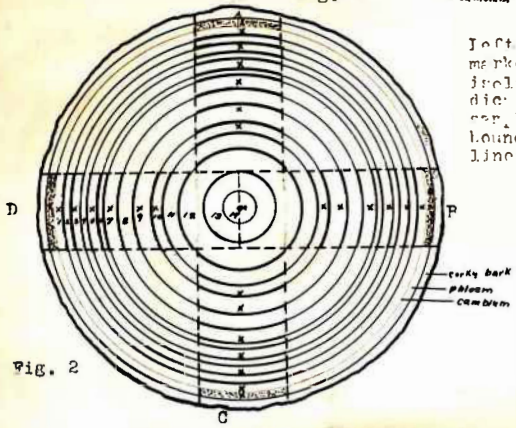


Fig. 2

C

Left: Cross section marked for fungus inoculations. (x) indicate points of sampling. Blocks bounded by broken lines cut out.

This long description of the technique of securing and identifying the fungi is given because we expect to use it next season, and the significance of the data collected would be entirely destroyed if the method were not trustworthy. The method was worked out in the preliminary work. It seems to promise the maximum protection against chance spores on the blocks and in the air, and at the same time the results from flamed blocks seem to indicate that the fungi in the phloem and in the interior of the blocks are not destroyed in the heating.

Thirteen beetle-attacked trees and five inoculated trees were sampled for fungi in the course of the season. About 50 plantings were made from each beetle-attacked tree studied, and about 700 plantings made in all. About 1000 cultures were handled during the course of the work.

Results:

The informal account of the results which follows will perhaps be more easily followed than a rigid presentation of the data.

It seems very probable that the spores of the bluestain fungi are carried on the bodies of the southern pine beetle and the secondary beetles, and that the trees are inoculated with these fungi by the beetles. A number of other forms have been isolated from the tissues of the trees, and presumably their spores may also be carried by the beetles.

Within a few days after the beetles open the bark and inoculate the tree; the first annual rings dry out, and do not conduct solutions. The bluestain can be observed growing in the phloem along the beetle galleries in scattered patches, and often in the first ring or two. The bluestain cannot, in the earliest stages examined, be seen next to the borderline between the conducting and non-conducting wood. The above condition has been observed in only about three trees which represented all of the earliest stages examined. In order to follow the course which a single pencil of sap would follow up the tree, the sections between the sampling points were taken to a mill and sawn vertically along the lines shown in Plate IV, page 31, fig. 2, connecting A-C and B-D. Examination showed instances in which the pencil of sap would not have had to pass through any apparent bluestain, but conduction had been stopped. Other cases were found in which the only observable bluestain which a pencil of sap would have

Fig. 1 Healthy Tree

Check # 14. Center of tree non-conducting, outer rings most active.

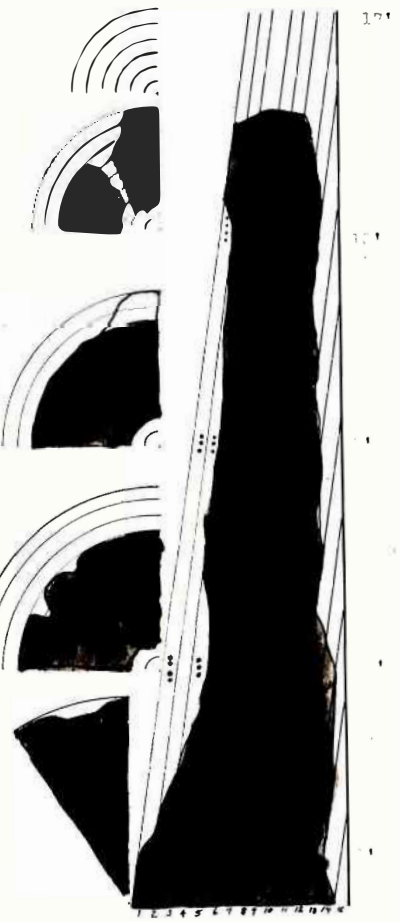


Fig. 2 Beetle-fungus Attacked Tree

Dendroctonus frontalis Tree # 77
Center of tree non-conducting; outer rings non-functional. No apparent blue-stain up the trunk. Ten days after attack.

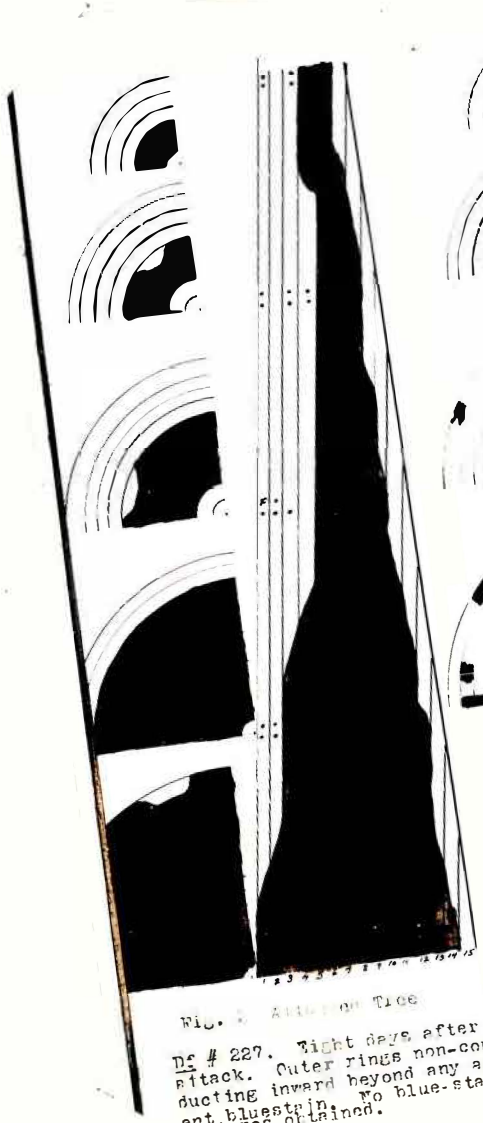


Fig. 3 Attack on Tree

Df # 227. Eight days after attack. Outer rings non-conducting inward beyond any apparent bluestain. No blue-stain cultures obtained.

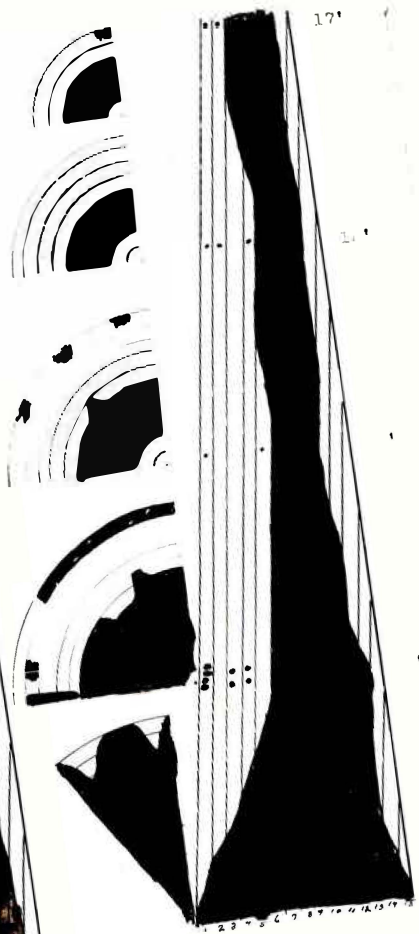


Fig. 4 Attack on Tree

Df # 229. Eight days after attack, but farther advanced than #227. No fungus cultures obtained from non-functional rings; scattered wedge bluestain above it if present, t

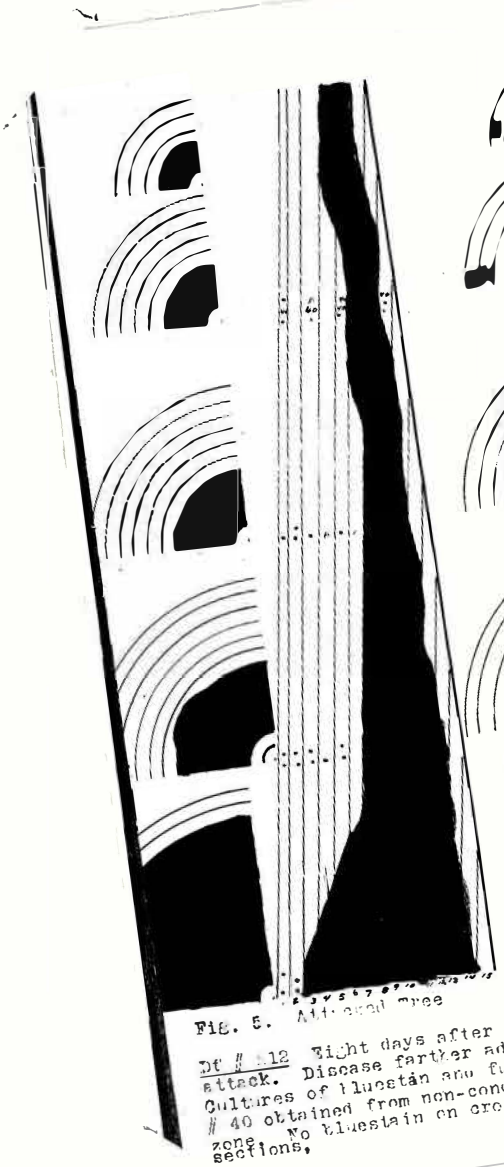


Fig. 5. Attacked tree

Df # 12 Eight days after attack. Disease farther advanced. Cultures of bluestain and fungus # 40 obtained from non-conducting zone. No bluestain on cross-sections.

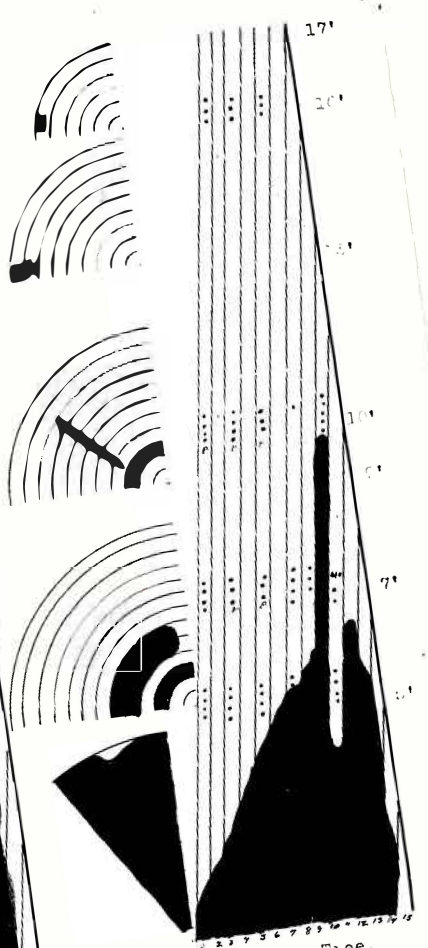


Fig. 6 Attacked tree

Df # 09 Eleven days after attack. Conduction of water probably cut off entirely at mid-crown. Considerable bluestain development, although not shown in cross-sections.

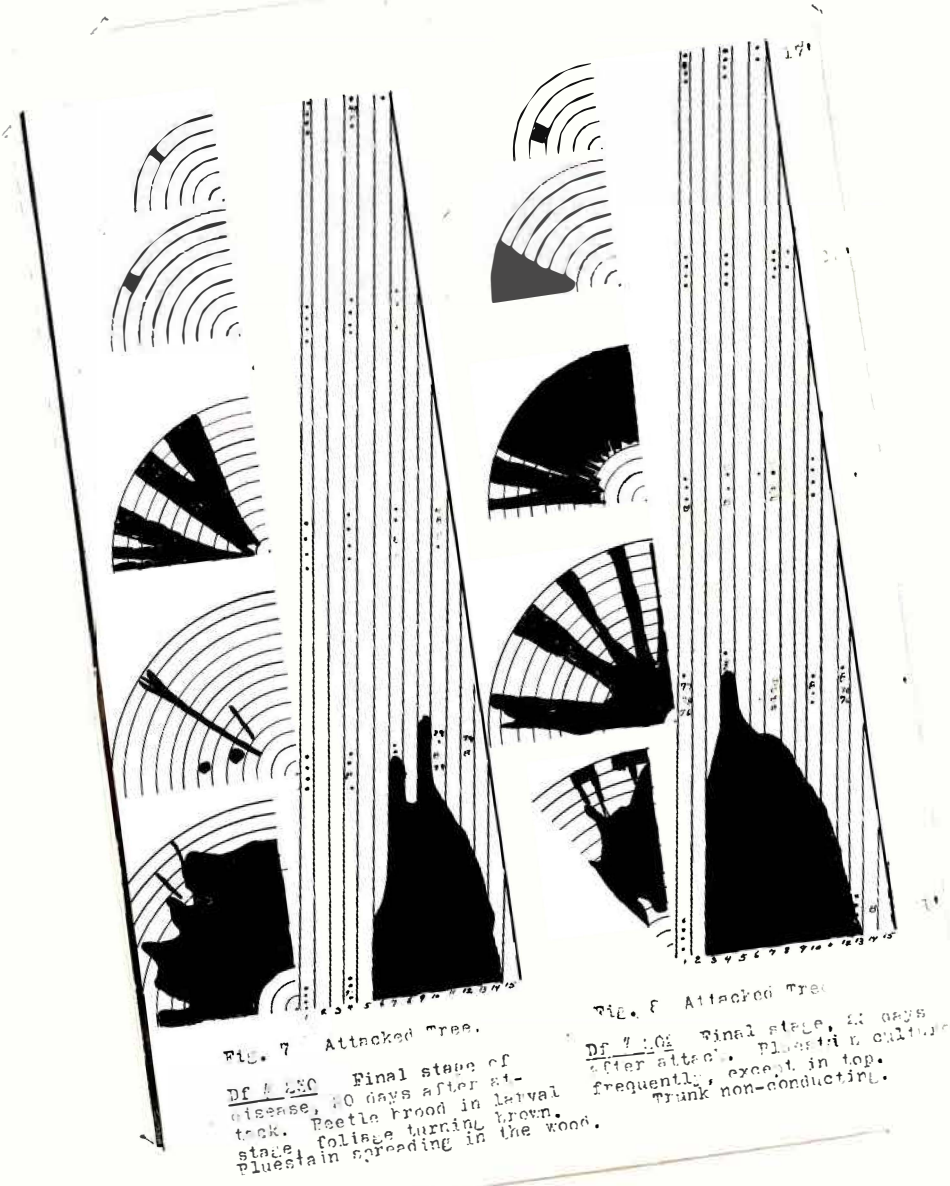


Fig. 7 Attacked tree.

Df A 230 Final stage of disease, 40 days after attack. Beetle brood in larval stage. foliage turning brown. Bluestain spreading in the wood.

Fig. 8 Attacked tree

Df 7 104 Final stage, 22 days after attack. Bluestain cultures frequently, except in top. Trunk non-conducting.

to pass were limited to the surface layer, whereas the dye was unable to pass up the wood farther in. Cases were also noted in which the pencil of sap would have to pass through apparent bluestain for only a few inches in the entire distance to the top, but was unable to pass up the rings beneath the wedge or above the wedge, or to diffuse laterally into these rings from the conducting zone. Inoculation experiments indicate that two inches of bluestain does not prevent the dye from appearing in the first rings for more than a few feet above or below the wedge, and this only after a long period. The most striking observation, which is brought out in the cross sections shown in Plate VII, page 38, was that the great development of the bluestain which is seen in dead trees does not occur until after conduction has practically ceased.

The questions at once arise as to how far the bluestain has penetrated beyond the apparent staining of the wood, and whether or not a fungus with hyaline hyphae may not be working in the tissues. The value of fungus culture work in answering these questions cannot be doubted, even though the results thus far are too meager to answer them.

In the culture work, it is assumed that the disease may develop more rapidly in some trees than in others. Figs.1-8, page 34, wereprepared to trace the general development of the diseased condition with relation to the conduction of solutions,and are based on an arrangement of the trees in or-

der of the development of the diseased condition. The cross section is shown, and the longitudinal section of the trunk. Only the rings along one radius are shown, as though the tree were sawn in quarters by a band saw. The bluestain is shown only on the cross section, which was taken from the actual maps of the cross sections. The vertical views are interpretations of the cross sections, and are only approximations to the true condition. The vertical sections are given to bring out the non-conducting center, and the shift in the dye stream from the outer rings in the healthy tree to the rings nearer the center.

In the early stages, the diseased condition of the outer rings interferes with vertical conduction, and with lateral conduction. Lateral conduction is indicated by the swinging of the dye stream to the outer rings above the non-conducting dried zone in Fig. 2. In this case there must be lateral conduction, but in most cases the dye might reach the particular position by vertical conduction, or lateral, or both. There has been time for considerable lateral diffusion or mass movement to take place, since these trees remained in the dye solution for three days.

The fungi obtained from the sampling points are indicated on the diagrams. The early stages are characterized by the few fungi encountered, and the failure to culture bluestain. Figure 2 has markings which indicate that no bluestain was cultured, and that fungus #40 was isolated from the conducting zone at 5 feet. This fungus was found at this height in 4 out of 17 plantings from the four radii, A,B,C, and D

as shown in Plate IV, fig. 2, page 31. For simplicity, the bacteria and yeast cultures are not indicated, so that a round dot indicates that the planting yielded no fungi or that only yeast or bacteria were obtained.

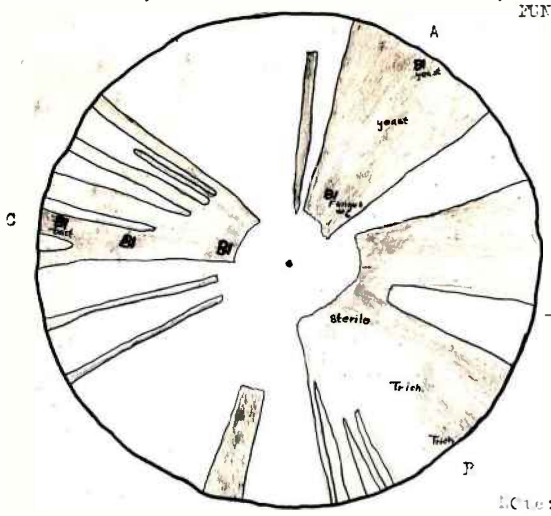
Bluestain was secured only from the phloem in Df229, shown in fig. 4. This is strange, since the fungus is certainly present. Bluestain cultures are not common until the final stages of the disease, shown in figs. 6, 7, and 8. Fig. 5 shows that bluestain cultures may be obtained, even though the cross section does not show the presence of the fungus. Bluestain is not the only fungus found in the interior of the tree, as an examination of the later stages of attack shows.

If instead of trying to follow a pencil of sap up the stem, cultures are made only from bluestain wedges, the bluestain fungus is almost invariably obtained. This is shown in Plate VII, page 37. Very frequently, pure cultures of bluestain are obtained, especially from the interior of the tree. The cultures obtained from the tree figured in Plate VII, page 38, are typical, except that Trichoderma sp. cultures were more commonly found in the other two trees investigated by this method. Where areas were taken which showed no bluestain on the surface of the section, Trichoderma sp. were commonly found, although occasional bluestain cultures were obtained. It was noted that bluestain could be seen in the interior of some blocks where it was not apparent on the surface.

PLATE VII

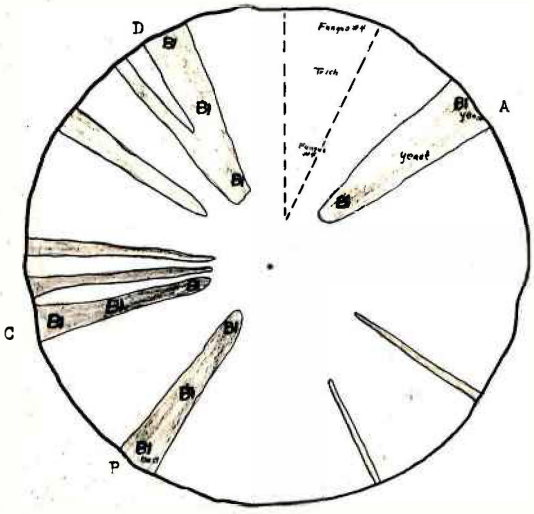
BLUESTAIN AT VARIOUS HEIGHTS, AND RESULTS OF FUNGUS SAMPLINGS

PLATE VII: BLUESTAIN AT VARIOUS HEIGHTS, AND RESULTS OF FUNGUS SAMPLINGS



C ft.
Pupae and larvae Dendroctonus frontalis

Note: This represents the results of making isolations from wood in the [illegible] stage of the disease. Pikh pine, 10 days after attack. Leaves yellow.



C ft.
Dendroctonus frontalis

PLATE VII

BLUESTAIN AT VARIOUS HEIGHTS, AND RESULTS OF FUNGUS SAMPLINGS

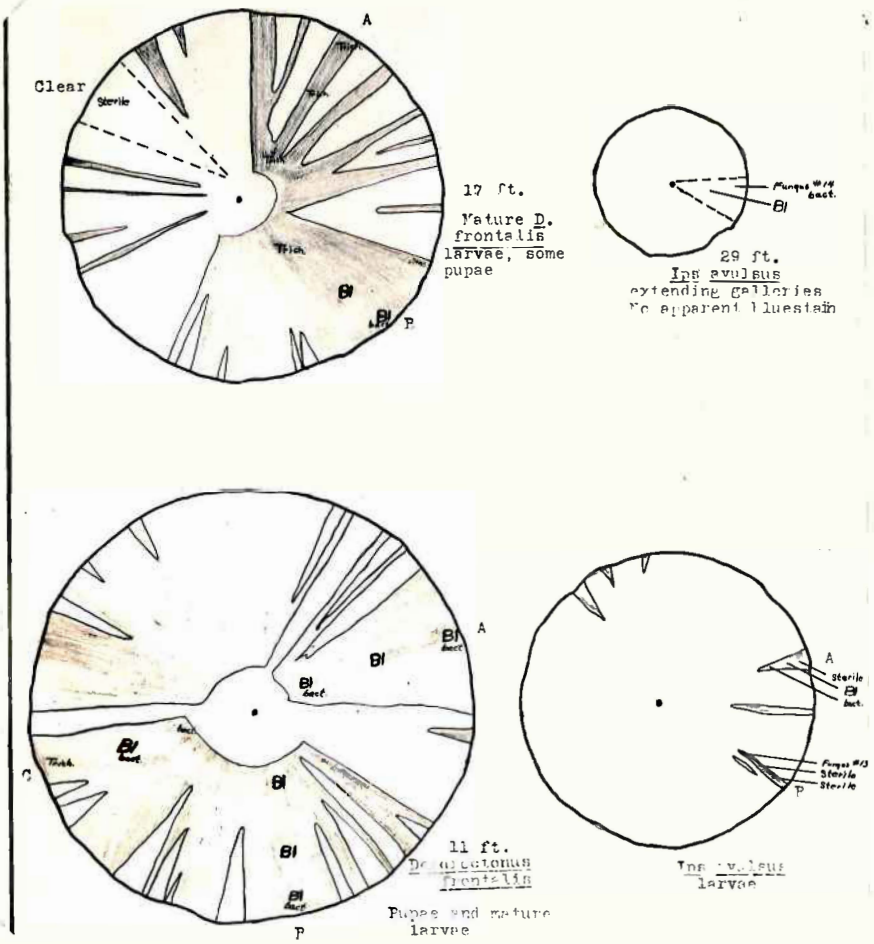
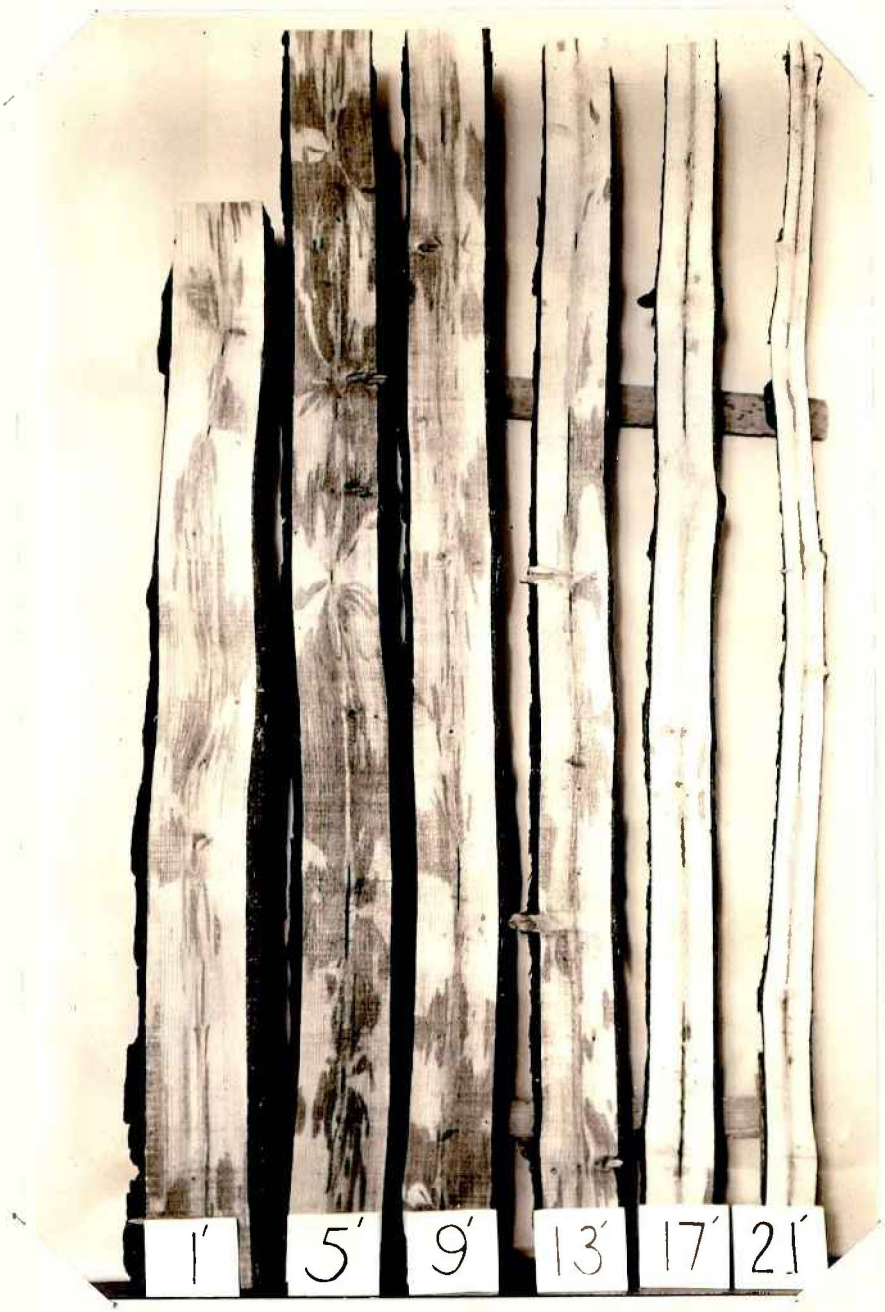


PLATE V



BLUESTAIN DEVELOPMENT

This represents a shortleaf pine 5 inches in diameter and 30 feet tall killed by the disease. The great development of the bluestain occurred after conduction had already stopped. Greatest development of bluestain from 5 to 13 feet. Leaves of crown entirely brown.

PLATE VI

LITTLE BLUESTAIN DEVELOPMENT

This tree was killed by the disease, but very little apparent bluestain was present. No bluestain below 9 feet, and little beetle attack below 9 feet. The beetles attacked high on the trunk. Water could reach the leaves without passing through a bluestain wedge, as far as bluestain is apparent. This suggests that simple drying due to opening of the bark galleries was the main factor in killing this tree.

The system of taking cultures only from bluestain wedges gives "consistent" results, but cannot be easily related to the sap stream, and cannot be used in early stages because little or no bluestain is apparent.

I regard this line of attack as one of the most useful of all tried. In my work with this disease I have noted the almost entire lack of bluestain in the early stages of attack, with at the same time a drying of the outer rings of the stem and the loss of conduction. If it is found that large areas of the trunk have no fungus working in them and at the same time are dried out and non-conducting, the opening of the trunk by the beetles galleries may account for a large share of the drying and non-conducting. It is true that a bluestain wedge probably can account for drying and stoppage of conduction at the point where it is growing, but a single wedge, obstructing a column of sap at the 17 foot level for a vertical distance of two inches up the stem, probably cannot account for the loss of conduction up the entire stem. This statement is based upon the experimental work which is described in the succeeding pages.

The data are interesting, but little more. It may be pointed out that the large number of cases in which sterile cultures were obtained from all stages may be due to the operation of chance. The sampling points are arbitrarily fixed, and it is a matter of chance if a bluestain wedge coincides with the sampling point. The bluestain and probably the other fungi occur in patches, which run together in the final stages of the disease. The patches of bluestain are shown in Plate VIII, page 43. The

merging of the bluestain wedges is shown in Plate VII, page 38, which represents a final stage of the disease.

PLATE VIII

BLUESTAIN PATCHES

The bluestain can be seen as dark patches on the trunk. This is a late stage. Water passing up the stem could not reach the leaves without passing through the wedges of bluestain, but the great development of bluestain took place after conduction had ceased.

no page 44

PART III

EXPERIMENTS

A number of experiments were tried during the 1931 season. Data secured from close description and in picturing the disease and in suggesting lines of attack, but they do not go much farther because of the number of factors which are operating. The experiments attempt to isolate the effects of the various factors which might be acting to cause the death of the trees.

1. Drying experiments

Purpose:

Although description tells us that water is being lost from the outer rings, it does not say how it leaves. Also, the fungus, bluestain, is found most abundantly in the region of drying. If the fungus could be induced to grow without drying occurring, and stoppage of conduction took place, we would be able to eliminate drying as a possible mechanism for the stoppage of conduction, at least in part.

Experiment I

Tree-topping Experiment

Purpose:

When the trunk of the tree dries, the water lost may be sucked out of the trunk by the transpiring leaves, or it may leave the wood through the beetle galleries and out the entrance and ventilation holes. Air may enter the wood by way of the galleries, or might conceivably be merely the

gases found in the center of the trees.

If the leaves are removed from a beetle-attacked tree, any moisture loss after that must be through the galleries (and cut ends of the branches.

Method:

A pitch pine (P. rigida Mill.) recently attacked by the southern pine beetle (about 3 days, galleries about 3 inches long) was selected and the limbs cut off close to the trunk up the entire stem, and the leader cut off. Care was taken that no needles remained on the trunk. A similar tree, attacked by the beetle and in the same stage, was selected as a check upon what would happen if the leaves were left on. A healthy tree was selected as a measure of what the tree might be expected to be like if it were not attacked by the beetle and not topped.

The trees were felled after 33 days, at which time the leaves of the beetle-attacked check tree, untopped, were turning yellow. Moisture determinations of the entire cross section were made at 3 foot intervals up the entire stem. The data are presented in Graph IX, page 45.

The trees in the experiment were about 30 feet tall and 4-5 inches in diameter at breast height.

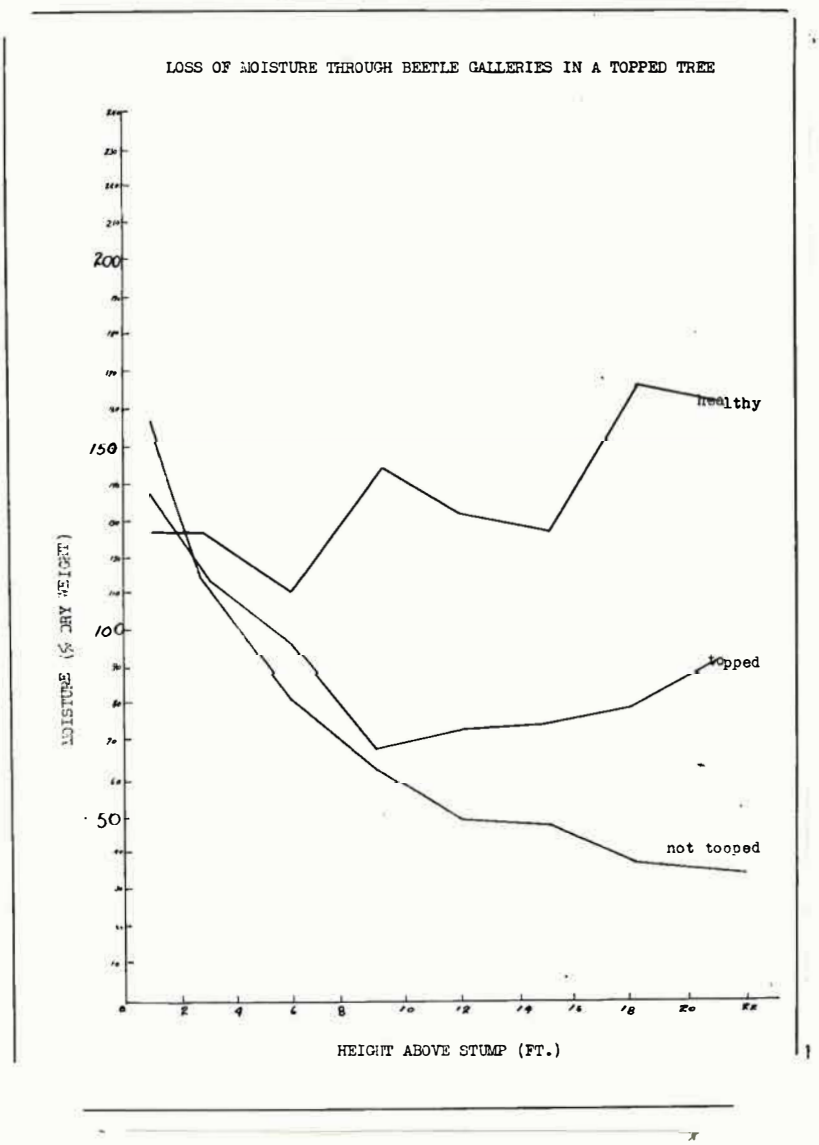
Results:

The beetle-attacked, topped tree has lost considerable

Begins to be
no p 47

GRAPH IX

LOSS OF MOISTURE THROUGH BEETLE GALLERIES IN A TOPPED TREE.



moisture, as shown by the comparison of the red line on the graph with the black line. The topped beetle-attacked tree has not fallen as low in moisture content as the beetle-attacked tree with the leaves left on.

Discussion: In this and the following experiments, the number of trees used is too small to make hard and fast conclusions. However, the results in this case are so striking that it probably can be repeated.

Conclusions

1. A single pine attacked by the southern pine beetle lost moisture through the entrances and ventilation holes of the beetle galleries, after the leaves had been removed.

2. No evidence is presented as to the relative amounts of water lost through the leaves and through the entrances to the galleries.

EXPERIMENT II

Waxed-bole Experiment

Purpose: If the beetle gallery entrance is necessary for the drying out of the trunk it should be possible to prevent drying by sealing the entrances to the galleries. (Also, if the bluestain fungus is able to continue penetration of the trunk after sealing the entrances, this sealing would eliminate drying along the galleries, and the effect of the fungus working alone in the wood could be determined.)

Method: A single shortleaf pine about four inches in diameter at breast height and 30 feet tall was selected for this treatment. The beetles had been working in the phloem only for

one or two days, and the galleries were about 1/2 to 3 inches long. No bluestain was apparent at the points examined.

The bar ridges were smoothed off with a knife and the infested length of the trunk covered with grafting wax made from beeswax, mutton tallow, and rosin. The wax was applied in a liquid condition with a paint brush. Although warm enough to be uncomfortable on the skin, the wax apparently did not injure the living phloem or the beetles, since the phloem remained white to the end of the experiment, and some of the beetles continued making galleries.

It was found after a few days that the beetles made openings through the wax and pushed out frass. These openings were filled with more wax on two occasions, but after that the openings were so infrequent that it was decided not to fill them.

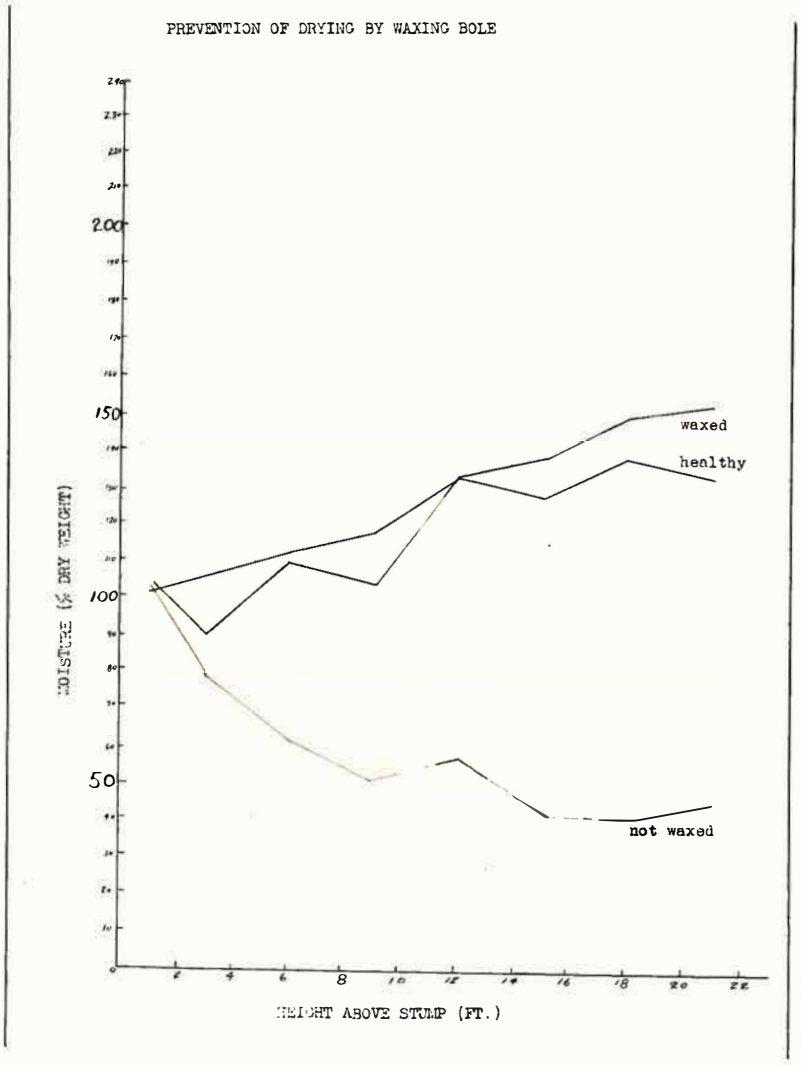
In order to determine what would happen if the tree were not coated with wax, a similar tree of approximately the same degree of attack, and in the same stage of the disease was selected. Also, a healthy tree of similar size and position in the canopy was selected for comparison. All trees were intermediates, by Graves' system of classifying trees. The trees were felled for analysis after 33 days.

Results: The data are presented in Graph X, page 51. The treated tree has almost the same moisture content as the healthy tree, and does not show at all the drying out of the attacked tree which was permitted to dry out.

Bluestain failed to develop; the phloem remained white and moist, and the galleries were not fully extended. The

GRAPH X

PREVENTION OF DRYING BY WAXING BOLE



foliage showed no change in color, when the tree which dried out had begun to turn yellow. The eggs of the beetles in the treated tree hatched in some cases, but the larvae failed to develop. The attacked tree which was untreated showed the usual brood development; mature larvae being present.

Discussion: As suspected would be the case at the beginning of the experiment, the conditions in the trunk were so changed from the usual course of the disease that the fungus was unable to develop.

Conclusions

1. A single tree in which the phloem had been tunneled by the beetles was prevented from drying out by sealing the entrances and ventilation holes leading into the galleries.

3. The air which takes the place of the water as drying goes on, probably enters by way of the openings to the galleries.

4. The beetle galleries and the beetles are a very important part of the disease picture.

EXPERIMENT III

Waxed-band Experiment

Purpose: If air enters the first few rings, it seemed logical that it might be sucked up toward the leaves due to the transpirational pull. Thus, the effect of drying at 5 feet might be the introduction of air into the transpiration stream at that point, with the consequent breaking of the ascending sap stream. According to this, the leaves would suck the water out of the stem and, instead of leaving a vacuum behind, air would be sucked up to take the place of the water. Air introduced at five feet might be sucked up to eight feet and effect conduction at that point.

Assuming that the air enters through the entrances to the galleries, if the entrances were stopped up for a space on the trunk, and there was a loss of moisture from the stopped area, the water would have to travel upward to leave the wood beneath the band, and the air taking the place of the water would have to come in below the band and be sucked up behind the broken sap stream.

The above argument is not entirely sound, but it gives the purpose of the experiment.

Method: A single pitch pine about five inches in diameter at

breast height was selected from a stand being attacked by the southern pine beetle. The beetles had been working in the tree for about three days, and had extended their galleries from 1/2 to 3 inches. The bark was smoothed in three bands around the tree, each band being 3 feet long. Alternate bands of untreated bark were left between the treated zones. The smoothed bark was coated with warm liquid grafting wax, applied with a paint-brush. The wax hardened immediately upon cooling.

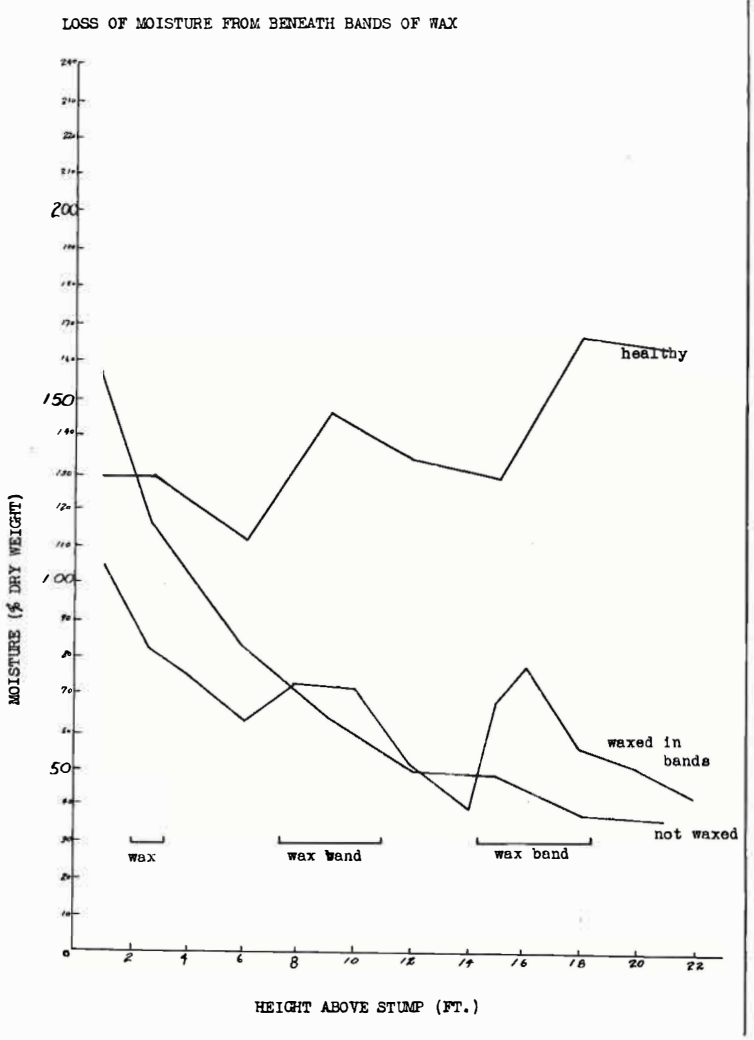
After 33 days, the tree was felled and moisture determinations made of whole cross sections of the wood at various heights. A beetle-attacked tree in the same stand and in the same condition of attack was selected as a check upon what would happen if the tree had received no treatment. A comparable healthy tree was also used for moisture comparisons.

Results: The moisture data are presented in Graph XI, page 55. The red line representing the treated tree shows that the banded zones coated with wax were less subject to drying than the uncoated parts of the trunk, but that there was a considerable loss of moisture from beneath the bands. If the tree had not been attacked by the beetles, the moisture content would have approximated the black line representing the healthy tree.

Discussion: The results are clear-cut enough to see that there has

GRAPH XI

LOSS OF MOISTURE FROM BENEATH BANDS OF WAX



been a loss of moisture from beneath the bands, but there is no certainty as to the path the water took in leaving the wood beneath the bands. It might be drawn up to the untreated zone and pass out the beetle galleries, or it might be drawn up the stem to the leaves. The air taking the place of the water might be drawn up beneath the band from below, or it might work its way down from above by diffusion, in the mutual exchange of molecules of water and air which might take place with the atmosphere.

Conclusion:

1. Water may travel vertically in beetle-attacked trees, as it appears to do in the case of this treated tree, but the whole trunk is opened by the beetles, so that the moisture need not travel vertically for an exit. That is, we still lack definite data as to whether water is sucked out of diseased trees by the leaves, or whether all of it leaves by way of the entrance galleries, or by way of both the leaves and the entrance galleries. The extremely rapid drying which occurs along the stem wherever the beetle opens the bark and inoculates the tree may be due to the rapid emptying of the outer rings by the leaves and the entrance of air into cells instead of water from below.

2. Inoculation Experiments.

Inoculation experiments permit us to work with the fungus away from any influence the beetles and beetle galleries might have in producing the diseased condition.

The general purpose of the inoculation experiments is to determine the conditions under which death occurs in inoculated trees, in order to compare them with the conditions actually obtaining in the beetle-attacked trees.

EXPERIMENT IV

Spiral Inoculation

Purpose:

1. To repeat in part the "classic" experiments of Nelson and Beal, in which it was shown that trees inoculated with bluestain died, and in which stoppage of conduction by the inoculated zone was demonstrated.

2. To determine the relationship between the advancing fungus and the stoppage of conduction in the tree.

3. To test if the spiral band of inoculum caused an accumulation of moisture below it in the trunk.

4. To discover the mechanism of the stoppage of conduction in inoculated trees, as a clew to the mechanism operating in beetle-attacked trees.

Method: The trees used in this experiment were all co-dominant shortleaf pines, and were about five inches in diameter at breast height and 30 feet tall. The ranges for the measure-

ments were 4.5-5.6 inches for the diameters, and 26-32 for the heights.

The cultures used in this experiment, and in the next experiment, were secured from Dr. Caroline T. Rumbold, Bureau of Plant Industry, from her cultures at the Forest Products Laboratory, Madison, Wisc. No attempt was made to check the identity of the fungus, or to commence work with a single-spore culture, or a culture from the tip of a growing hypha. Dependence was placed upon the identification given by Dr. Rumbold, as follows:

"Culture # 187-314 5/26/31 (date of transfer) Ceratostomella pini from Pinus echinata infested with Dendroctonus frontalis. Collected by R. M. Nelson, April 1, 1929, at Asheville, N. C." Six cultures were supplied, and each new lot of rice was inoculated with the fungus from a culture which had previously been unopened.

Twelve trees were used in this experiment, divided according to the following treatments:

Spiral Band Inoculations

1. Bluestain on rice
2. Rice alone
3. Trichoderma on rice

The Trichoderma sp. culture was obtained from a beetle-infested tree. It was thought that this fungus would help to keep unknown fungi out of the rice, and a better comparison with the bluestain inoculation would be obtained. Trichoderma apparently does not injure the trees. The rice used alone, without being inoculated with any fungus, was contaminated

from the air in the process of applying it, since no effort was made to work under asceptic conditions.

In order to secure a spiral band about the tree, the bark, including the phloem, was removed in a spiral band about 2 1/2 inches wide, each turn of which was one foot from the next. This band is shown in Plate IX, page 63, and five turns about the tree, extending from three feet above the ground to eight feet. Thus it was expected that it would be possible to determine the number of times it would be necessary for a band of bluestain 2 1/2 inches wide to cut the ascending sap stream before conduction was stopped.

The set of trees inoculated with Trichoderma was not started until two weeks after the first two sets were treated, due to delay in securing the fungus.

Results:

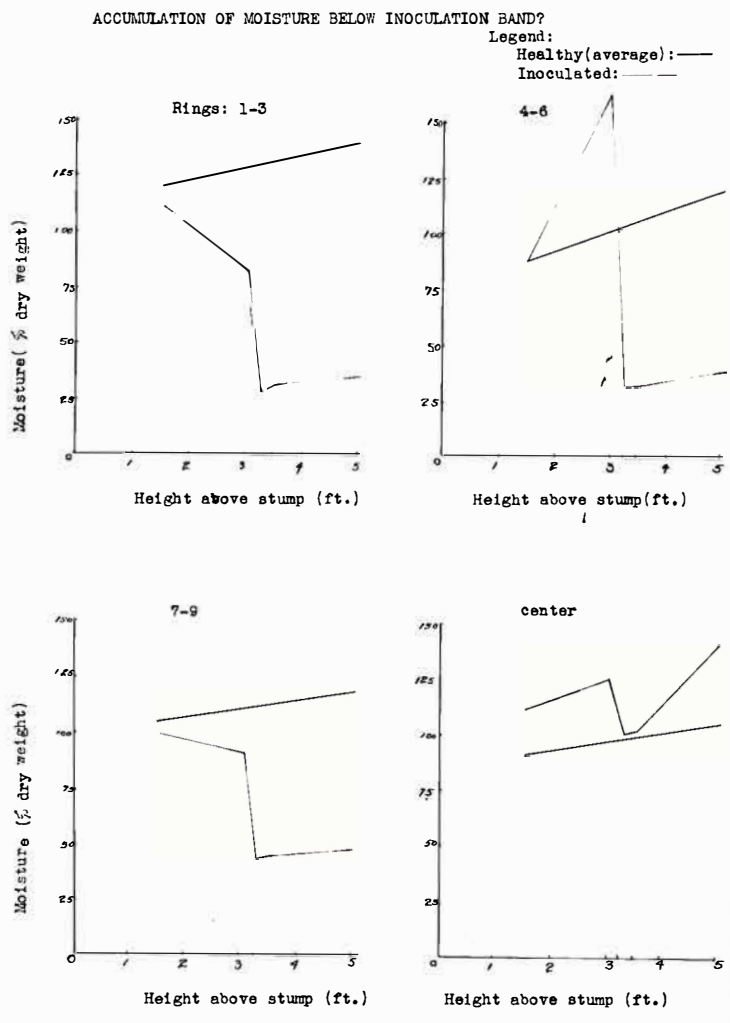
a. moisture relationships

It soon became apparent that no satisfactory information could be obtained concerning the moisture relations and the stoppage of conduction, since the effect of each turn of the spiral affected, or might affect, the one above or below it. Also, it was necessary to cut the trunk on a slant to secure as much of the spiral as possible. This made chipping difficult. For this reason, moisture data were taken only on a single tree, shown in Plate IX, page 63, figure 1. The data are shown in Graph XII, page 60, in which a healthy tree is compared with the inoculated tree. The healthy tree represents an average of all data on healthy trees.

in the figures in Pl. VIII, page 63. The spiral marks

GRAPH XII

ACCUMULATION OF MOISTURE BELOW INOCULATION BAND?



-no page 61

It has been suggested that the moisture content is higher below a bluestain wedge than at the wedge or above it. This is explained on the basis of a "damming" effect by the bluestain wedge. The test of this point is whether the moisture content is higher below the band than a corresponding point in a healthy tree. In the case of the tree examined, only the 4-6 rings are significantly higher than the average healthy tree. The next experiment gives more data on this point, which will be taken up then..

b. Fungus isolations from the inoculated zones.

In order to determine if the fungus applied in the rice was the one actually penetrating the tree, an effort was made to recover the fungus from the trunk of the tree. The results from the bluestain inoculated trees (IN 1 and 2) and the check trees which had only rice applied to them (IN 5) are given below: (Bl; bluestain; F, fungus; contam., contaminated; Ster., sterile)

Bluestain Inoculated

Tree #	Ht. up tree	Annual ring from which sample was taken				
		3	6	9	10	
IN1	3'	Bl	Bl	Bl	Bl (contam.)	
	5'	Bl	Bl	Bl	Bl	
	7'	Bl	Ster.	Bl&F		
IN2 (wedge)	Ht. 4'	1	3	6	9	10	11
A		bact.	Bl	Bl	Bl	Bl	Bl
B		Bl	Bl&F	Bl	Bl	Bl	Bl
D		F	Bl	Bl	Bl	Bl	Bl
A	7'	All cultures sterile. (No penetration of bluestain) 8 taken					

Rice inoculated

IN5 All cultures sterile, 10 taken.

PLATE IX

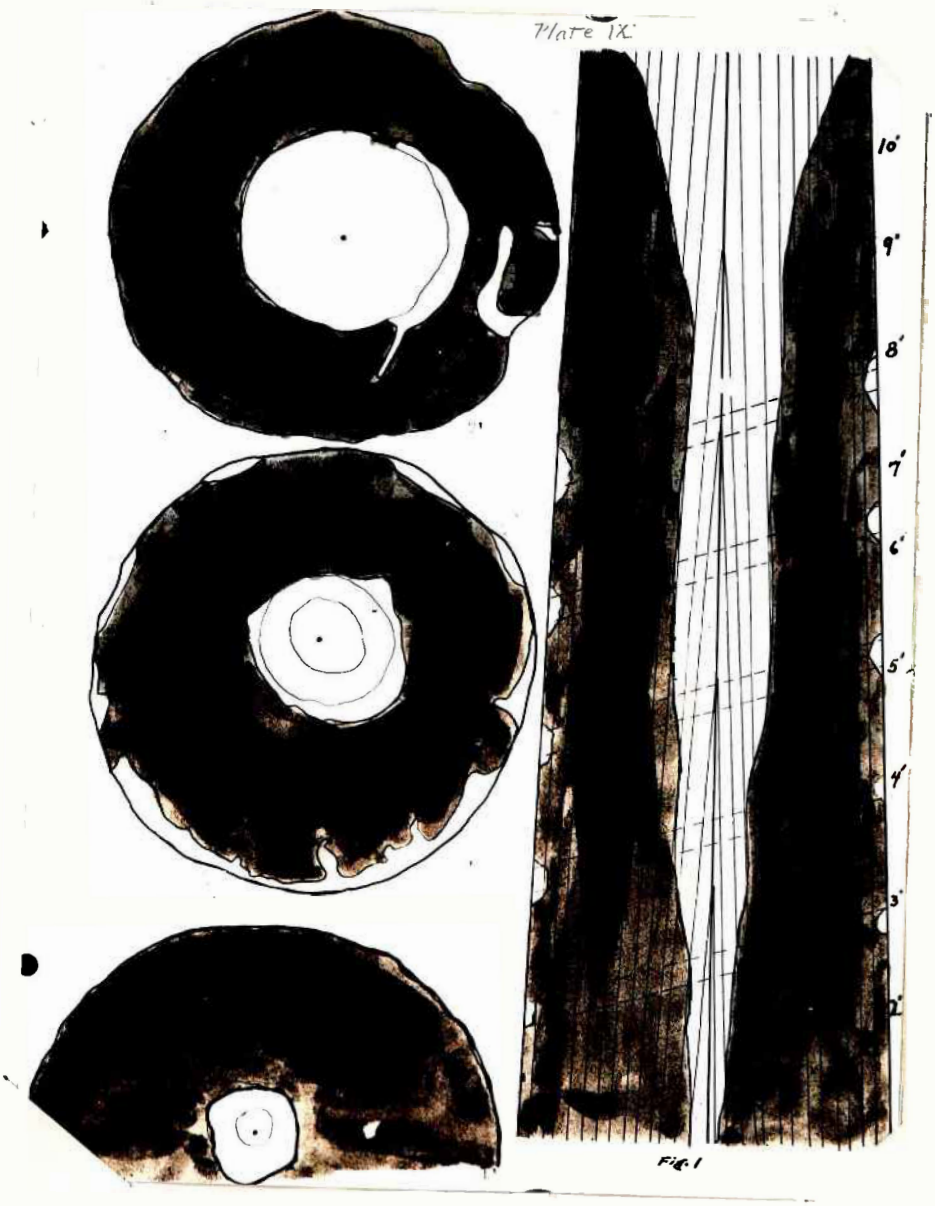


PLATE IX

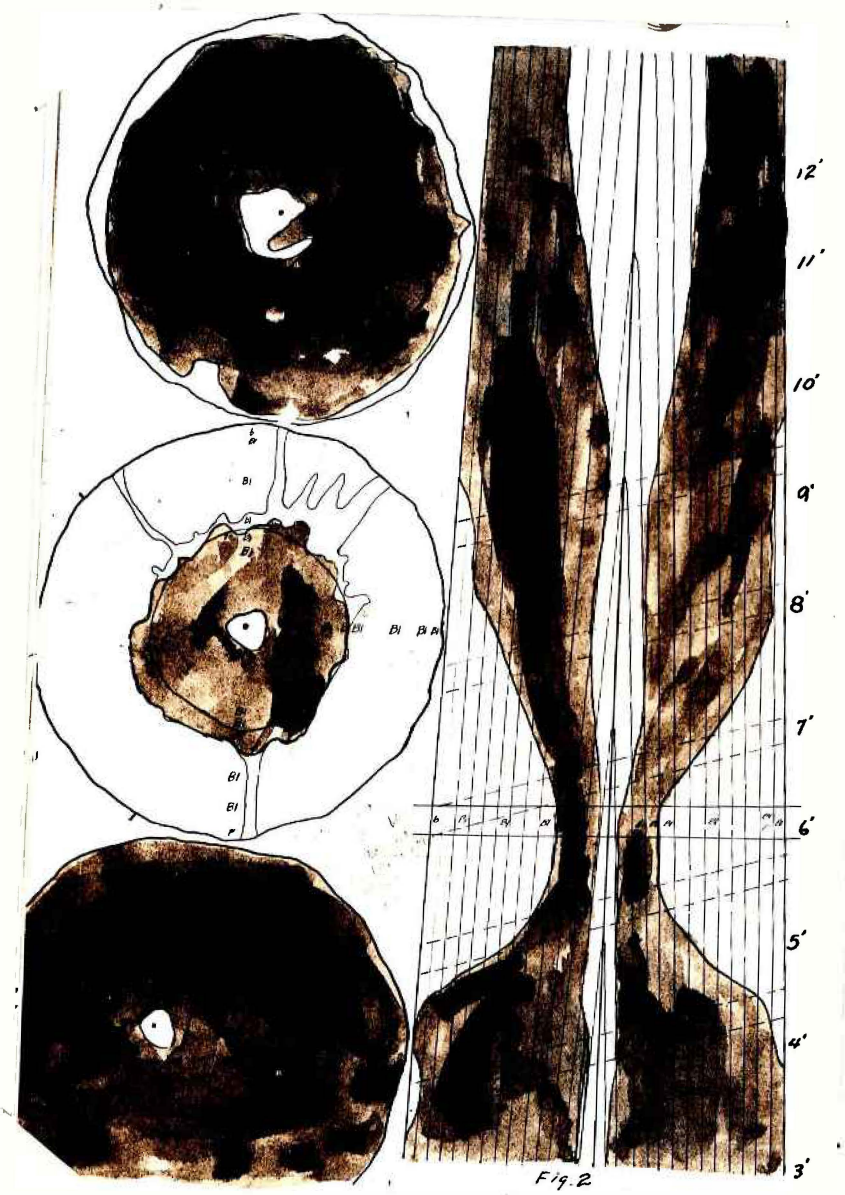


Fig. 2

Summary Isolations

The bluestain fungus appears to be the only fungus consistently isolated. Other fungi are only rarely encountered, and then only in the outer rings, with one exception. The check tree upon which rice was applied appeared to be sterile in the wood.

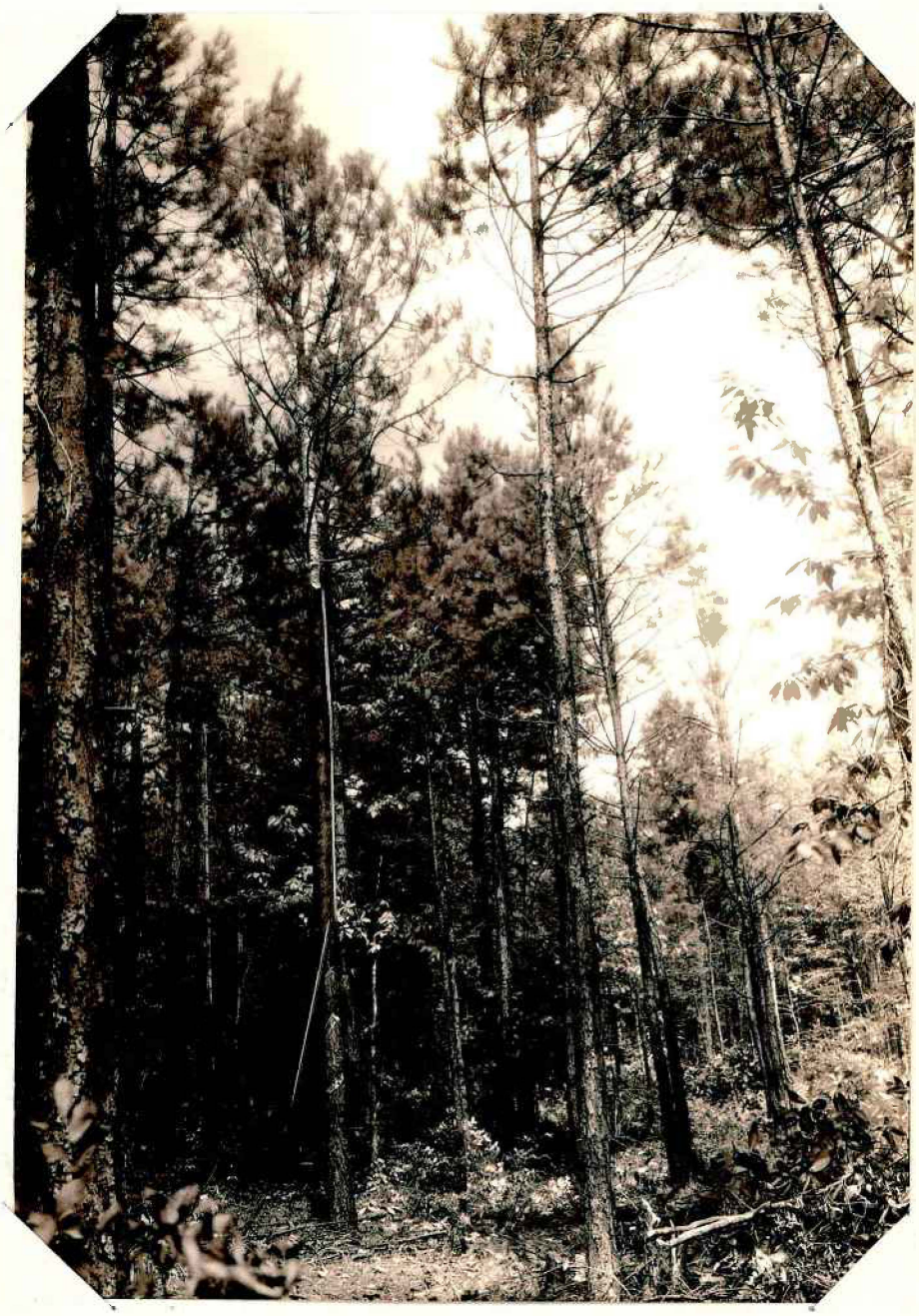
c. Death of the inoculated trees

When it was apparent that the method of inoculation was not suited to moisture and conduction studies, interest centered upon the death of the tree.

In order to study the stages of death, the trees were stepped into dye solutions at various lengths of time after inoculation. It was apparent from the first that the conduction was being progressively cut off as the fungus advanced into the tree at the inoculated zone. A final stage was secured when the leaves were turning yellow. This was 36 days after inoculation. The 1929 and 1930 needles were yellowing, and the 1931 fading. No attempt was made to analyze the situation except to note that no dye was taken up the tree and that the upper part of the trunk appeared very light and dry. The tree was sawn up the center, and found to be stained by the fungus entirely across the trunk, at the inoculation zone. A control tree, upon which rice had been applied, was not affected as to conduction except the first few rings in the injured zone where the bark had been removed.

A tree was left 60 days, at which time the entire top was brown. This tree is shown in Plate IX, page 65.

PLATE IX



BLUESTAIN-KILLED PINE

A shortleaf pine inoculated by the spiral method with bluestain. Top brown after 60 days. Wire screen used to keep out beetles. Wrapped with cloth in upper part.

The method of protecting the inoculated trees from insects is also shown in this picture. When the picture was taken the trees inoculated with rice and with Trichoderma were apparently healthy.

Summary of experiment

1. Trees inoculated with a culture of bluestain died, the trees losing the power to conduct solutions in the inoculated zone progressively from the surface layers of the wood to the center of the trunk.

2. Control trees treated in the same way with rice and with Trichoderma on rice were apparently unaffected, as far as could be judged.

3. No good evidence of a "damming" action by the bluestain was obtained, but the evidence against such an action was weakened by the occurrence of a single case which might be interpreted as a "damming" of the solution.

4. The leaves apparently emptied the trunks of the killed trees of a large amount of the water: [Since the trees were protected against insect attack.] If so, the leaves possibly sucked up air after the sap stream was broken.

EXPERIMENT V

Band Inoculations

After the spiral inoculation method failed, it was decided to limit the bluestain zone to a short band about the tree. In this way it was expected that the factors which cause the stoppage of conduction would be concentrated in a narrow band, and that their effects on the conduction in the stem could be more easily determined.

Purpose:

1. To determine the amount of bluestained wood necessary to kill a tree.

2. To determine the relationship between the advancing fungus and the stoppage of conduction.

3. To test if moisture accumulated below the band.

4. To determine the conditions under which conduction is stopped, as a clew to what happens in beetle-attacked trees.

(5. To get the fungus to grow without the wood drying out, in order to separate the fungus from the drying.) The experiment failed with respect to #5, since drying took place in every case.

Methods:

Eight shortleaf pines were chosen from the same stand in which the descriptive work given in Part II was done. These trees were co-dominants, and average 32 feet in height and 5 inches in diameter at breast height. Plate X, page 68 illustrates the stand and also shows the points on thetrees

PLATE X

BAND INOCULATION

Tree inoculated with bluestain at 12 feet in a four inch band about tree. Bark replaced, and inoculated band coated with wax and waterproofed cloth to prevent drying of the inoculation band.

where the inoculation band was placed. The height of the bands shown is about 12 feet.

A strip of bark was removed to the cambium in a band about 4 inches wide around the trunk. The exposed wood was given a generous coating of rice in which bluestain was growing in pure culture. The bark was then tacked back in place. Four of the eight trees were given the additional treatment of covering the replaced bark with wax, in order to prevent drying of the wood. No rice or Trichoderma "check" was used.(1)

Results:

The treated trees were given casual inspections at various times up until 30 days after treatment, when it was discovered that the secondary bark beetle, Ips calligraphus Germ., was attacking certain trees. Only the trees which had no grafting wax over the inoculation zones were being attacked at that time. Since this beetle, like the southern pine beetle, introduces bluestain fungi, it was apparent that if anything was to be secured from the work, it would be necessary to examine the trees as to conduction at once, in order to be able to distinguish the effects of the inoculation.

Examination of the wood at the band showed no bluestain penetration, and an accumulation of resin crystals, on the surface of the wood beneath the rice, which had dried out. Two trees were stepped in dye solutions 25 days after inoculation. These are shown in Plate XI, figs. 2 and 3.

(1) Mr. Buckenhahler and Mr. Bygant made the inoculations.

two more trees were stopped in dye solutions 30 days after inoculation. To tests if the entire tree would die, two trees were screened against insect attack. The remaining two trees were lost, due to insect attack, although examination of the inoculated band showed a condition similar to the bands on the other trees.

Data were taken on 4 of the 8 trees, according to the following headings:

(1) Fungus isolations from the inoculated bands
(2) Distribution of dye solutions
(3) Moisture gradient, by rings
(4) Air content

(1) Fungus isolations from the inoculated bands

Although no bluestain wedges were apparent to the eye, it was necessary to test for their presence by making plantings from the inoculation zone. The same technique was used as in the analysis of beetle-attacked trees. The relationship between the position of the fungus and the conducting rings is shown in Plate XI, figs. 2 and 3. The table below gives the results of the plantings.

Legend:

Bl, bluestain
F, fungus
O, sterile
-, no planting made

Plantings from Inoculated Bands

Tree #	Wedge	Rings from which samples were taken						
		1	3	5	7	9	10	11
IN 14	A	O	Bl	O	Bl	O	-	O
	B	O	O	Bl	Bl	O	-	Bl
	C	O	O	Bl	O	O	-	O
	D	F	O	O	O	O	-	O
IN 19	A	F	O	O	Bl	O	-	O
	B	O	Bl	O	O	O		
	C	Bl	Bl	Bl	Bl	O		
	D	O	Bl	Bl	Bl	O		
IN 16	A	-	Bl	Bl	Bl	O		
	B	-	Bl	O	O	O		
	C	-	Bl	Bl	O	O		
	D	-	Bl	O	O	O		
IN 20	A	O	O	O	O	O	O	
	B	O	O	O	O	O	O(contaminated)	
	C	O	O	O	O	O	O	
	D	O	O	O	O	O	O	

The table and the chart seem to show that bluestain is the only fungus which has entered the wood, and that it is not found in the cultures from IN 20. We think the data are interesting, but inadequate. In four out of thirteen cases bluestain is present in the ring just before the conducting ring. In the remaining eight cases, no such relationship seems to exist. (It must be kept in mind that the bluestain is proven to be present when a culture of it is secured, but it is not nearly so certain that it is absent if no culture is obtained. The best safeguard against the chance of missing the fungus is to make numerous cultures at close intervals.)

PLATE XI

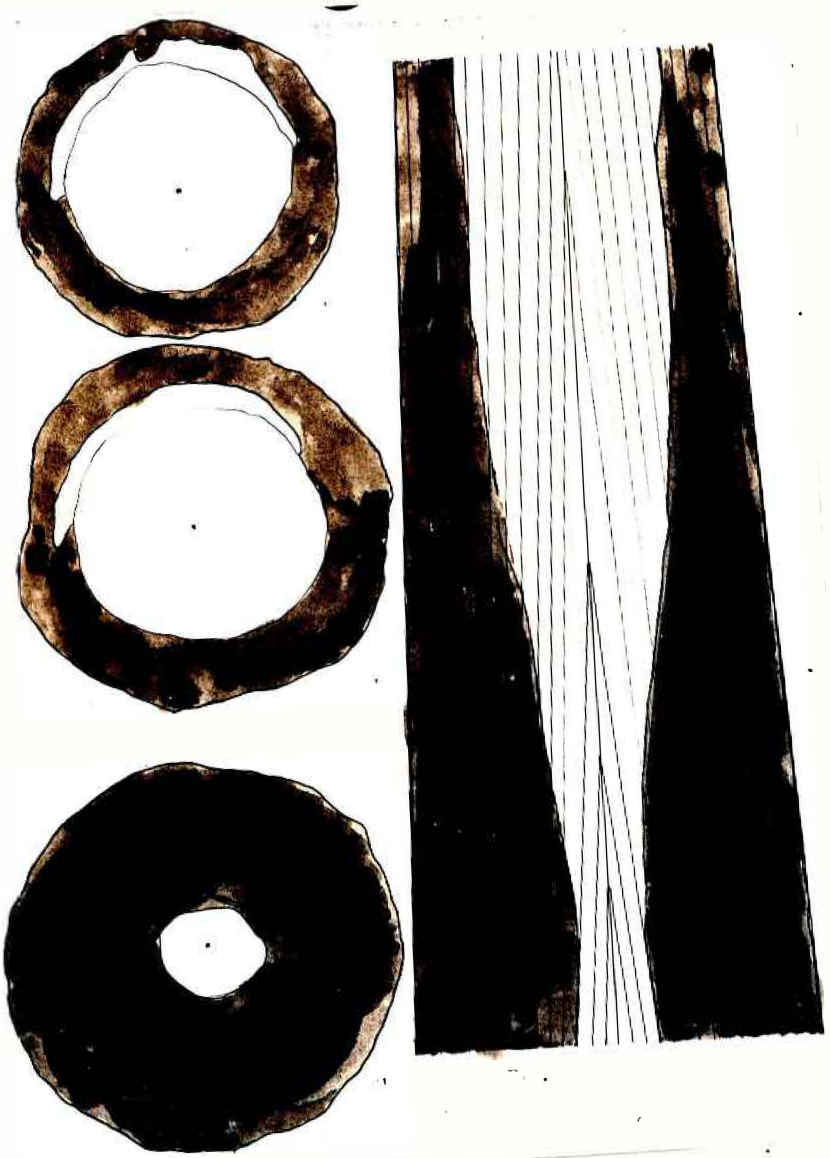


PLATE XI

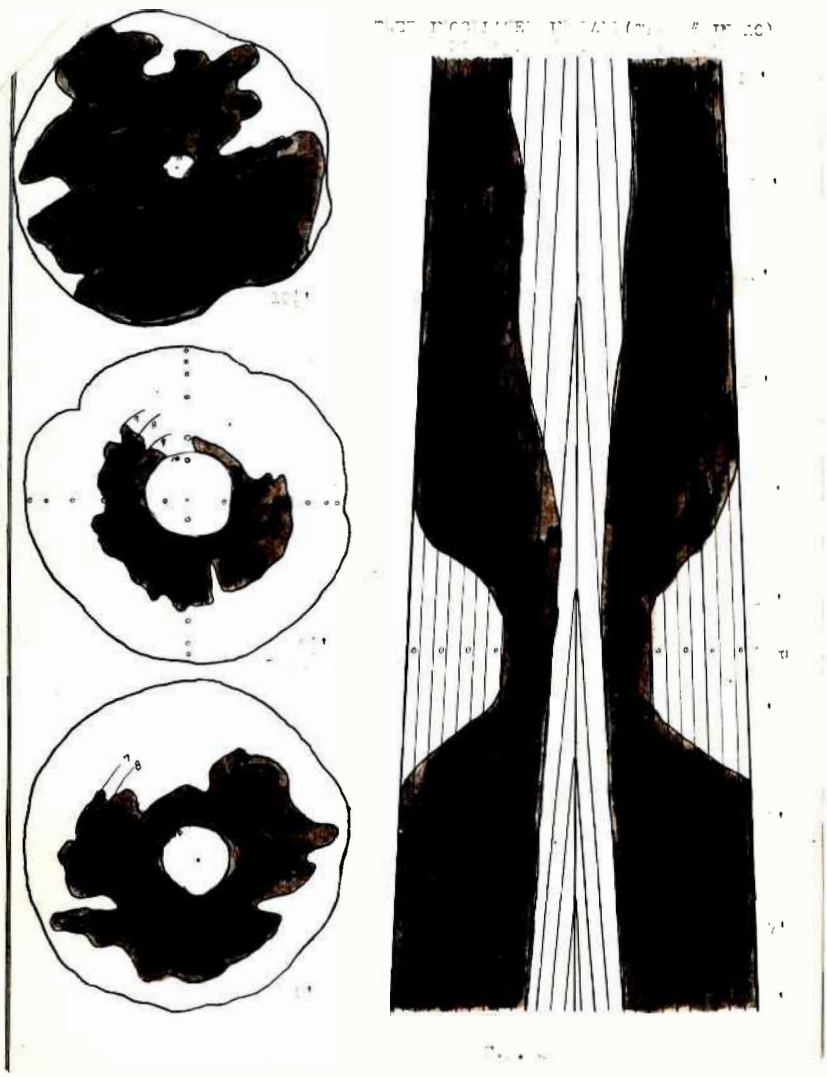
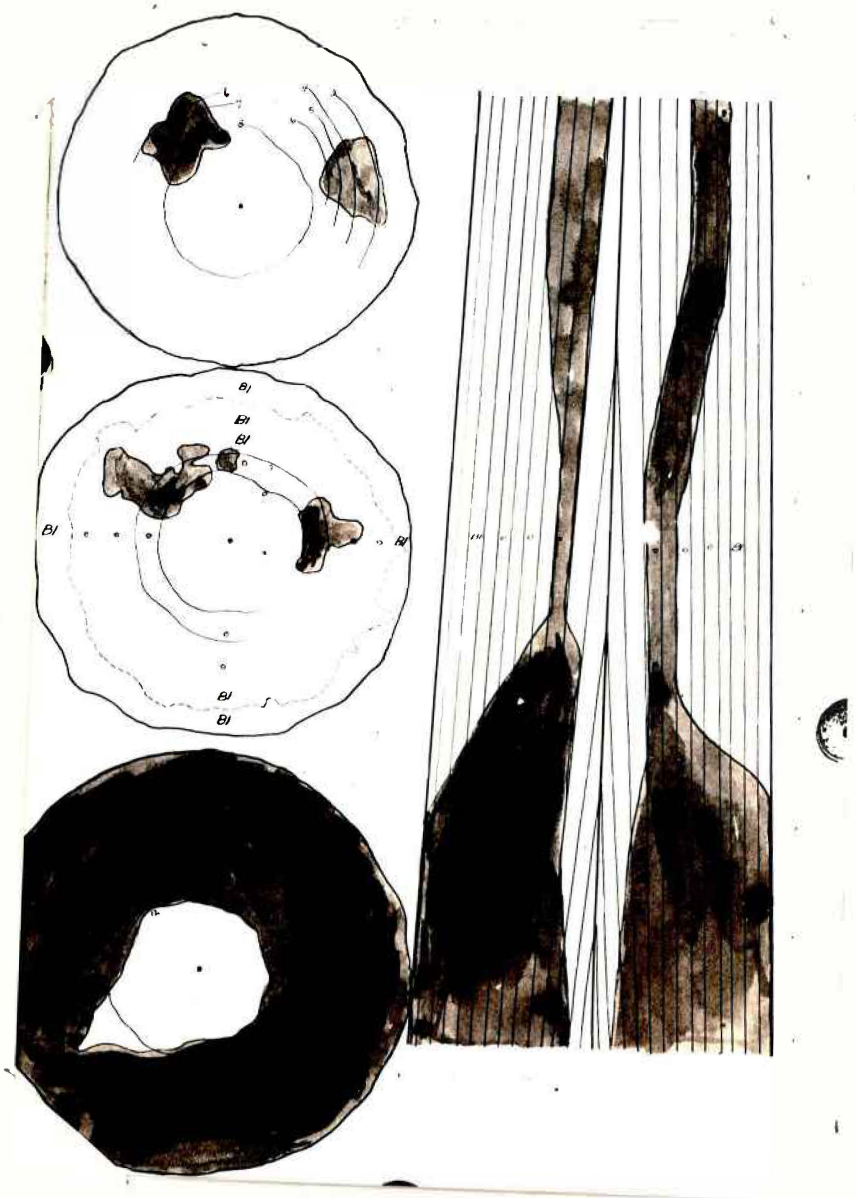


PLATE XI



(2) Drying at the inoculated band

The most striking effect of the inoculations was the drying of the band areas. The effect of the inoculation and drying upon conduction is shown in Graph XIII, page 74. (Left conduction data off for simplicity.)

The localization of the drying is well shown in Graph XIII, A, B, C, and D. We suggest that the advance of the drying zone into the tree is sufficient to account for the stoppage of conduction and the consequent death of the tree. It is possible that no fungus had entered IN 20, as indicated by the fungus isolations. If so, parallel cases might be set up for the beetle attacked tree and the inoculated tree:

DEATH DUE TO
STOPPAGE OF CONDUCTION

brought about by

exposure of the wood to the air by

A. (beetle-attacked)	B. (inoculated)
beetles making galleries	removal of phloem in band
subsequent drying due to	
1. simple evaporation and transpiration	1. simple evaporation and transpiration
or	or
2. penetration of bluestain	2. penetration of bluestain
or both	or both

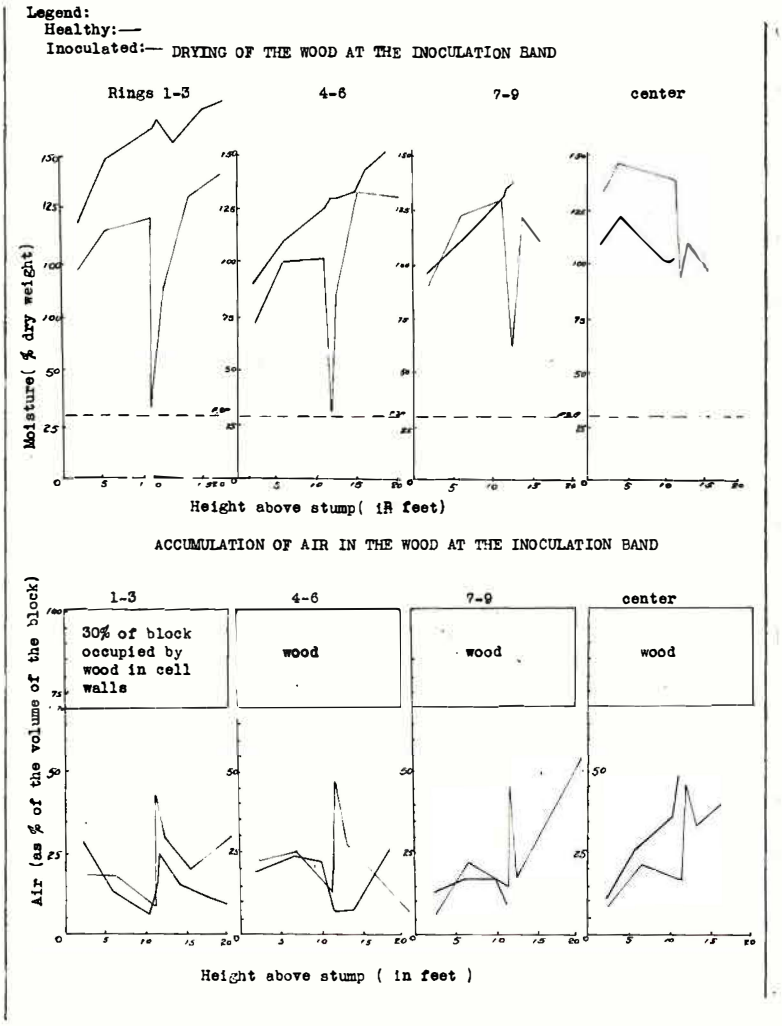
There must be, in the case of the inoculation band, a critical point at which under the conditions, the free water air ratio becomes such that conduction no longer takes place, if drying of the wood is causally connected with stoppage of conduction. Not sufficient work has been

GRAPH XIII

DRYING OF THE WOOD AT THE INOCULATION BAND

ACCUMULATION OF AIR IN THE WOOD AT THE INOCULATION BAND

GRAPH XIII 74.



done to determine if such a critical point exists or what it might be for the trees in this stand. However, the gradation from the rings with low moisture content and high air content which do not conduct, to the rings with high moisture content and low air content, which do conduct, can be shown for the study. Graph XIV, (A), gives all of the data for the inoculated zones. The critical line is set at the point representing the moisture content of 75% of the dry weight. This is done because for one tree, the conducting area was cut out and found to have this moisture content; since it was only a small part of the section, and probably on the verge of becoming non-conducting, it is selected to represent the critical point. This is not greatly at variance with the data secured from beetle-attacked trees: (--See Gr. VIII, p. 25); a line drawn at 75% on this graph (VIII) would separate the conducting and non-conducting fairly well. Of course, more work is needed before any of these approximations have any significance.

(3) Accumulation of air at the band.

The drying of the band is accompanied by an accumulation of air. This is shown in Graph XIII, page 74, E, F. G, and H. The massing of the data for the inoculated zones is given on Graph XIV, page 76, (B). The critical line might perhaps be drawn at 45% of the volume of the block, or about 55% of the volume of the lumina, if we assume that the wood substance occupies about 30% of the block. Since there may be a considerable error in our approximation, there is no inconsistency in the suggested water air ratio at the stoppage-of-conduction point in beetle-attacked trees of 60/40

GRAPH XIV

AIR-MOISTURE RELATIONS AT THE INOCULATED ZONES OF INOCULATED TREES

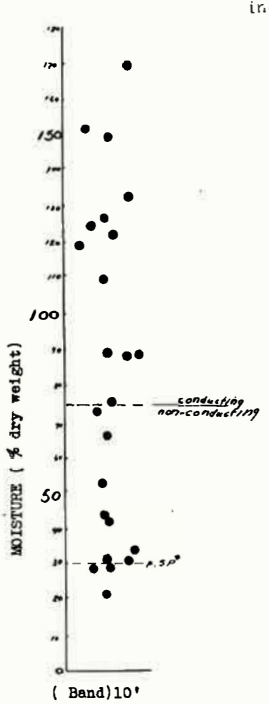
GRAPH XIV 76.

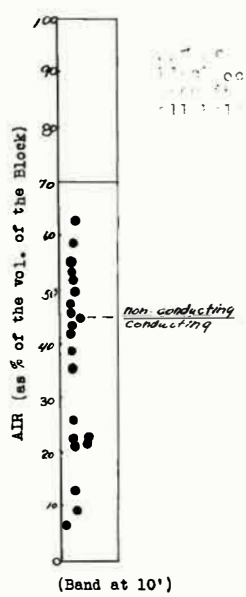
AIR-MOISTURE RELATIONS AT THE INOCULATED ZONES OF INOCULATED TREES

Legend:
Conducting: (capable of conducting [illegible], but not actually having [illegible])
Non-conducting:
Partially cond.:

Note: All determinations for the various girdling groups, on the live, of the healthy trees in addition, are included in the determinations below.

A





and that for the inoculated tree of 55/45.

(4) Accumulation of moisture below the bands

In two cases out of 15, there is a significantly higher moisture content below a band than at the same point on the healthy tree. Although this prevents a real elimination of the idea that moisture is "dammed" below the bluestain wedges and inoculation bands, the theory is weakened.

Summary and Conclusions

1. An inoculation band of 4" inches about the tree at aheight of ten feet, is sufficient to kill the tree. (The trees kept to test if the tree would die had turned yellow after 60days)

2. The relationship between the advancing fungus and the stoppage of conduction is not clear; sometimes it was found next to the conducting ring, and at other times not. In one case, no fungi were isolated from the inoculation band.

3. The evidence is against the accumulation of moisture below the band,but exceptions occur.

4. Apparently, in these trees, conduction halts at the same time the air H_2O ratio in the lumina is about 55/45 %; this is not inconsistent with the 40%/60% ratio assumed for the beetle-attacked trees.

3. Interception-cut Experiments

In the last section describing the band inoculations, it was found that the dye stream avoided the inoculation zone, and that the effect of the inoculation zone was felt above and below the band for about a foot, in the case of two of the trees. (Plate II, fig. 2, page 72). Although the water content was not low, no dye appeared in the outer rings. There might be several reasons for this. An attempt was made to cut out a section of the band equal to the dried area of the band, but the experiment failed. The experiment below is the only good evidence we have thus far on the ability of the dye solution to diffuse laterally to the surface beneath a girdle removing several rings of wood. This experiment was performed in 1930.

EXPERIMENT VI

Interception-cut Experiment (2)

Purpose:

1. To find the effect of removing the outer rings of wood for a short distance on the trunk on the distribution of dye solutions.

2. To try to have the tree suck up air, and see if this stops conduction.

Methods: This work was done in the same stand used in the 1931 work, on inferior trees. The intercepting cuts shown in

(2) Experiment performed for me by Mr. Hugo Pawek of the University of Minnesota.

PLATE XII

INTERCEPTION CUT EXPERIMENT 1930

PLATE XII 79.
INTERCEPTION CUT EXPERIMENT 1930

Fig. 1 The removal of the first two annual rings ½ way around tree at 5' does not prevent the dye from appearing above the cut and below it in the outer rings.

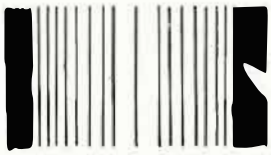


Fig. 2. A knife cut through the fourth ring ½ around tree does not prevent the dye from reaching the first ring above and below the cut.

Note: In the inoculation bend experiments, it was found that the dye did not come out to the surface again until about a foot above the bend, and did not appear at the surface for a foot below the dry zone. The moisture content of these points, however, was as high as the healthy. The diagrams tend to indicate that the dye might be expected at the surface immediately beneath the dry zone, unless some other factor were acting to prevent it from diffusing outward.

Fig. 3 An axe cut 8-10 rings deep did not prevent the dye from appearing in the first ring at the surface above and below.

Plate XII, page 79, were made at breast height. The three methods of making the cuts are illustrated. Some of the cuts were covered with wax, others were left exposed. The results were the same, hence only the exposed cuts are illustrated.

Results:

1. The dye appeared in the outer rings above and below the cuts.

2. The dye passed the cuts, and probably moved laterally to the outer rings.

Conclusions:

1. The removal of the outer rings in the form of a band for a short distance on the stem does not prevent the dye from appearing in the outer rings above and below the bands. A cut removing the first, first 2, and first 4 outer rings was not sufficient to stop conduction above and below the band.

2. The bands covered with resin, and the tree failed to suck up air from the cuts which were left exposed.

4. Sterile Beetle Experiment

EXPERIMENT VI

If the beetle could be freed of the attendant fungi, it might be induced to gallery the trees, and thus we might be able to separate the effect of the galleries from the effect of the fungus.

Method:

Various solutions were used to sterilize the beetles, eggs, and larvae. These were alcohol, copper sulphate, and mercuric chloride. Various strengths were used, and various lengths of time for dipping, until a combination of solution and time of dipping was secured which sterilized the surface of the eggs, but did not kill them.

It was found that larvae commonly had only yeast or bacteria associated with them in the egg niches. An attempt was made to destroy the fungi which might be along the fresh beetle galleries, in order to have the eggs hatch in galleries with only the yeast or bacteria present. The method used was unsuccessful.

Results

1. Sterile larvae were secured by dipping the eggs into alcohol and mercuric chloride solutions. Some of the larvae remained alive for 10 to 15 days, but failed to grow on malt agar. Others planted in living phloem on wood sections also failed to develop. No attempt was made to work out an artificial medium for the larvae.

2. This description does not do justice to the experiment. A great deal of time and effort was given to the

problem, and careful records kept. Leads were discovered and technique developed which will prove invaluable if the project is taken up again. This experiment was the most interesting of all the experiments undertaken.

PART I

METHODS USED IN TREE ANALYSIS

In the 1931 work, a beginning was made on the determination of the errors involved in the methods of sampling used. These methods and the errors involved are described and discussed in Part I. The following discussion becomes more intelligible if the main body of the report beginning with Part II, page 1, is read first. This will also obviate much repetition.

1. Moisture sampling of the wood

In order to trace the manner in which the beetle-attacked trees dry out, the moisture per cent. of dry weight in the successive rings from the outer rings to the center of the tree at various intervals up the tree was determined. The grouping of the annual rings in the 1930 work was (inclusive) 1-2, 3-5, and the remaining center. This was changed in 1931 to give larger samples and a more detailed description to (inclusive) 1-3, 4-6, 7-9, and the remaining center rings.

The chipping method of sampling was used. This consists simply of removing the successive groups of rings from a cross section of the trunk with a sharp knife. A section about two inches thick (Fig. 2, Plate I, page 5) was cut from the felled tree at the desired height. The groups of rings were removed by striking down on the back of a hunting knife held so as to chip off the groups. The final trimming was done with a clasp-knife, and the fine shavings discarded when only moisture

samples were being made. The time required to remove a single sample of three rings around the circumference of the cross section was about eight minutes.

The method used for determining the green weight of the sample was to weigh the cross section before any wood was removed, and to weigh the cross section again after the rings were chipped off. The difference between the weights was taken as the green weight of the sample. Although certainly not free from error due to evaporation and loss of chips, this method of determining the green weight of the chips does away with a large part of the error due to the evaporation of moisture from the fine shavings.

In the 1930 work, the green weight of the chips was determined by weighing the chips after they were removed from the cross section, rather than weighing the cross section. To determine the error involved, the two methods for determining the green weight were compared. The cross section was weighed before the rings were chipped off and after chipping, and the chips themselves were weighed. The 1930 method is in error by the amount of moisture it lost during chipping. This error was computed as the per cent. the loss made of the higher wet weight secured by weighing the cross section. The average error for 13 determinations was 6.4%.

2. Method of drying samples

The moisture determinations for the 1930 season were made

in a Sargent water-jacketed oven in which the temperature during drying varied from about 95 to 105 degrees centigrade, although at one time the temperature on one set of samples went up to about 120 degrees for a short time. Repeated weighings were made of nine representative samples from different parts of the oven until the variation after at least eight hours heating since the previous weighing was .1 gram or less. This usually took three days, and the average difference in weight was much less than .1 gram.

Better ovens were available for the 1931 determinations. A Freas electric oven capable of regulation within about three degrees above and below 100 degrees centigrade was used for one set of samples. Unfortunately, the oven was improperly regulated, and the temperature mounted to at least 128 degrees during the night. The graphs for the trees affected by this heating do not show any remarkable variations.

All other samples were dried in a large Freas electric oven equipped with a fan and capable of maintaining the temperature constantly within at least a degree of the desired temperature. The inside dimensions of the oven were 36 x 18 x 24 inches. The temperature was regulated to 100 degrees centigrade. The samples were dried until successive weighings after at least eight hours had elapsed differed by .04 grams or less. Three days were usually taken for drying, although this was probably longer than necessary for most samples. Six samples of the heaviest weights in different parts of the

oven were selected for the trial weighings.

3. Volume determinations

In the 1931 work it was desired to measure the area included in the groupings of the annual rings, and to measure the length of the cross section from which the rings were to be cut. This was necessary in order to compute the volume of the wood removed. A planimeter was used to measure the areas of the ring groups on the cross section of the trunk, and a centimeter rule divided into 1/2 millimeters was used to measure the length of the cross section. The operation of the planimeter is so familiar that its operation is not described here. The ring groups of the cross section were planimetered directly by tracing the rings through a pane of glass placed over the section. Later a thin sheet of celluloid was substituted for the glass. The areas desired were traced three times and an average taken. The length of the cross section was secured by taking an average of eight measurements spaced at regular intervals around the circumference.

The areas of the groups of rings(1-3, 4-6, 7-9, and center) were measured before the corky bark was removed. The length of time required to planimeter a medium-sized cross section was about 10 minutes. The amount of moisture lost from the section during this time was small; an average of eight determinations gave 0.1% of the original weight of the cross section lost.

The volume of wood removed in chipping is assumed to be

the same as that secured by measurement, which of course is not the case. In order to determine the error involved in accepting the measured volume in place of the actual volume removed, the volume of the rings was first determined by measurement, and then by immersion in water. In the latter case, the volume of the cross section was determined before and after chipping the sample, and the difference between the determinations taken as the volume of the rings removed.

The following paragraphs from Technical Note Number B-14, issued by the Forest Products Laboratory, Madison, Wisconsin, was followed in determining the volume of the cross section by immersion.

"The volume of the specimen may be found by determining the weight of water it displaces when immersed. This weight in grams is numerically equal to the volume of the specimen in cubic centimeters.

A container holding water enough for complete submergence of the specimen is placed on one pan of a balance scale. The combined weight of the container and water is then balanced with weights added to the other scale pan. By means of a sharp rod the specimen is held completely submerged without touching the container while the scales are again balanced. The weight which is added to restore balance is the weight of water displaced by the specimen, and if in grams is numerically equal to the volume of the specimen in cubic centimeters".

This determination was carried out for only a few samples. Further work on this point has been postponed until next season. The immersion method gave volume determinations lower by about 2.5% of the measured volume. The actual error may very well be larger than this; the data show a number of gross errors probably due to lack of skill in using the planimeter.

The errors in determining the volume of wood removed in planimetering are probably the largest experimental errors involved in the measurements, and are especially important since they appear as air volume in the final calculations.

4. Beetle galleries

An attempt was made in the 1931 work to secure a better estimate of the amount of phloem occupied by beetle galleries. The area of beetles galleries within a band of bark around the tree was determined by measuring the length of the galleries and multiplying this figure by the average width of the galleries. To secure a sample of the bark, a cross section of the tree was cut off at the desired height so as to give a strip of bark around the trunk about two inches wide. The ridges of corky bark were cut off, and the remaining corky bark and tunneled phloem removed in as large pieces as possible. It was often possible to remove a whole band about the cross section without breaking it. The strip of bark was placed beneath a pane of glass on which inch squares were ruled. The length of the galleries falling within a whole number of squares, usually ten, was measured by running the wheel of the planimeter along them on the glass, and reading the figures on the graduated wheel of the planimeter. The figure on the graduated wheel corresponding to a measured distance of ten inches was divided into the figures corresponding to the length of the beetle galleries, in order to convert the length into inches.

The average diameter of a beetle gallery was determined

by measuring the diameter at ten places with an instrument known as a core-measuring device. This is a moving stage under a binocular microscope. The distance between two points is determined by placing the object on the stage beneath the microscope, focusing a hair line on one of the points, and then moving the stage by means of a small crank until the second point is beneath the line. The distance moved by the stage is registered on a dial in hundredths of a millimeter. An average of 20 widths of Dendroctonus frontalis galleries gave 1.72 millimeters. Ips avulsus galleries measured 1.07 millimeters.

Tests were made of the errors involved in other methods of sampling than those used in this study. These methods are important because they offer a means of studying the standing tree throughout the course of the disease, and because they are used in other tree studies.

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5. The increment borer method.

This method involves simply the boring into the tree with a Pressler increment borer to the desired depth, removing the core, and either taking the whole core as a sample or clipping the rings desired from it. A number of cores are generally taken in order to increase the size of

the sample. In the work described in the following discussion, the cores were placed in small vials with tightly fitted corks.

a. Increment core compared with the cross section

The test trees used in this and the succeeding comparison were dominant shortleaf pines about thirty feet tall and six inches in diameter at breast height. Four borings were made at each sampling point, and a cross section taken for comparison.

(In the treatment of the comparisons between methods of sampling, the method giving the higher value is considered the correct one. The determination for the other method is generally slightly above or below this line, which is considered the zero line in all cases. In choosing the "correct" method, it is considered that the method with the largest amount of material in it is more likely to approach the true value than the very small sample.

The difference, positive or negative, of the method being tested from the "correct" value, is taken as a measure of the reliability of the method. The variation of the method being tested from the accepted values is plotted on the graphs, with the "correct" values taken as the zero line. This does away with the effect of the moisture gradients in the tree upon the appearance of the graph, at least to a large extent. In most cases the divergence from the correct values is plotted interms of the differences in the respec-

tive dry weight percentages. This approximates the per cent. error curves quite closely. In order to avoid further delay in the presentation of the report, these curves are included in their present form instead of preparing new curves.)

If the increment borer method is compared with the cross section method for obtaining the average per cent. of moisture per unit dry weight in the trunk of a tree at a given point, there is strikingly slight variation in the actual values obtained. This is shown by Graph XV, page 92. In this graph the per cent. errors are used, instead of the difference in the percentages, since this graph was made up later than the first ones.

TABLE I

INCREMENT BORER METHOD COMPARED WITH CROSS SECTION METHOD OF MOISTURE SAMPLING.

Determination #	1	2	3.	4	5	6	7
per cent. Error	+1.1	+3.3	-10.2	-1.3	-5.0	-2.3	-6.9
Deviation from cross section, in % units	+1.2	+4.1	-12.7	-1.6	-6.9	-3.5	-13.4

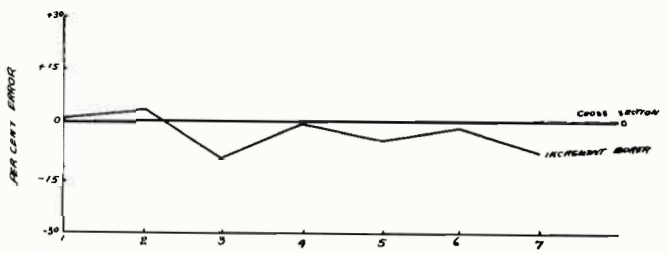
The data indicate to us that the increment bore method gives a low value for the moisture content of the cross section. If determinations #3 and #7 are considered as of unlikely occurrence, the values are fairly consistently in error

GRAPH XV

PER CENT POSITIVE AND NEGATIVE ERROR INVOLVED IN USING THE INCREMENT BORER IN PLACE OF THE CROSS SECTION

GRAPH XV

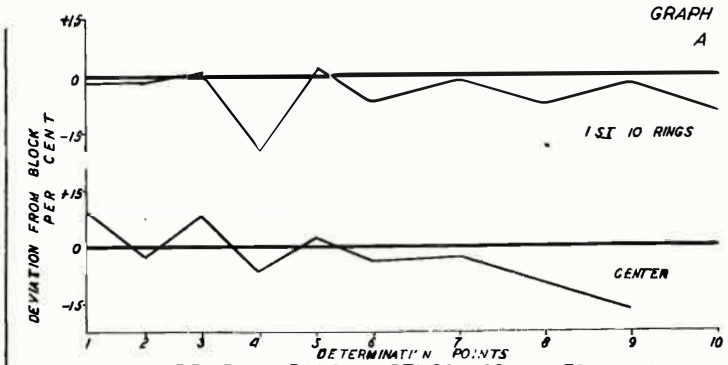
PER CENT. POSITIVE AND NEGATIVE ERROR INVOLVED IN USING THE INCREMENT BORER IN PLACE OF THE CROSS SECTION



Determination points

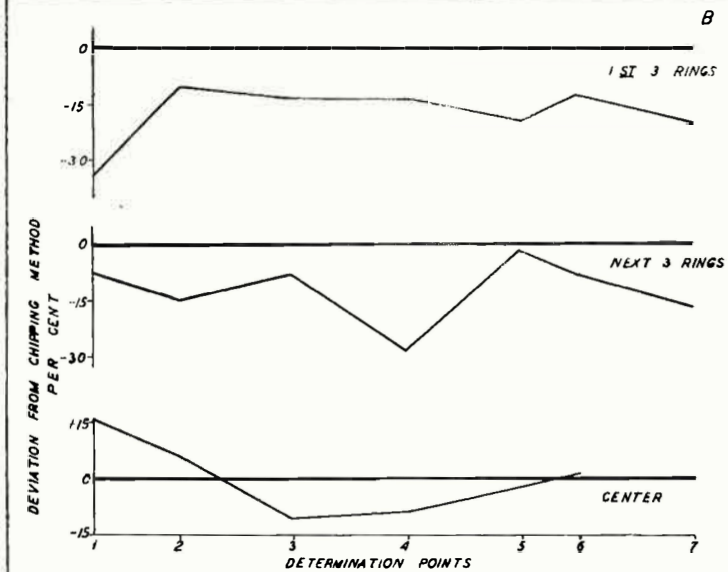
Data from T. Tree # 182.

GRAPH 16



INCREMENT BORER METHOD COMPARED WITH BLOCK METHOD OF MOISTURE SAMPLING

1—5 EX TREE NO 3
6—10 EX TREE NO 4



INCREMENT BORER METHOD COMPARED WITH CHIPPING METHOD OF MOISTURE SAMPLING

by a rather small amount.

b. Increment borer method compared with chipping method.

If it could be shown that the increment borer could be used in sampling the various groups of rings, we would be able to follow the drying-out of a single tree. This would avoid one of the variations in the present method, in which the disease is followed in different trees.

The increment borer method was tested against the chipping method by taking samples by the two methods from the same sampling point. The increment cores were taken to the center of the tree and the core extracted. The first three outer annual rings of growth were cut off, then the next three, and the remaining core to the center was taken as the third sample. Four such cores were taken about the trunk at each sampling point. To compare with these, the same rings were chipped off with a knife.

From a consideration of Graph 16, B, page 93, we conclude that the increment borer gives a fairly consistent error when compared with the chipping method as the Zero line. The graphs for the "next 3 rings" and the center are not nearly as regular as that for the "1st 2 rings"; but further work would undoubtedly improve these curves. Grouping all data, the average amount to which the negative values differ from the "correct" values is 12.2%.

c. Increment borer method compared with the block method.

In order to further test the increment borer method,

GRAPH 17.

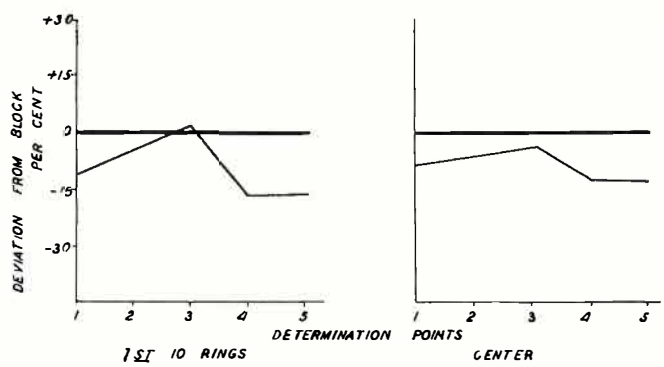
95.

GRAPH 17
A

AUGER METHOD COMPARED WITH CROSS SECTION METHOD OF MOISTURE SAMPLING

1–6 EX. TREE NO. 1
7–11 EX. TREE NO. 2

B



AUGER METHOD COMPARED WITH BLOCK METHOD OF MOISTURE SAMPLING

samples were taken of the first ten annual rings and the remaining center by the increment borer method and by simply cutting out a block containing these rings. The sizes of the blocks for the first ten rings and the remaining center was determined by the width of the rings, and a distance along the rings of about one inch.

The results of this comparison are presented graphically in graph 16, A. Here the block is taken as the zero line, and the deviations of the increment core from it are graphed as positive or negative according to whether they are higher or lower than the block values. The error involved in using the increment borer appears to be fairly consistent. The values for the increment borer tend to run low, averaging 6.6% for the negative values.

6. Auger borer and cross section methods compared.

The ordinary carpenter's brace with a one-inch bit is a tool which has some advantages in securing moisture samples from standing trees. It gives a large sample in a short time, and can be operated more or less easily from a ladder. Its chief disadvantages are that it is difficult to take samples of specified groups of rings, and the error involved in its use is an unknown quantity, generally assumed to be a large one.

In order to determine this error, samples were secured by making two bores completely across the trunk of the

felled test tree, at right angles to each other but at different levels to avoid their intersecting. The borings were caught in a small paper bag held beneath the opening of the bore hole.

The determinations of moisture from samples secured by the auger are almost invariably low, and in the determinations made, there was no regularity in the error. This is shown in Graph 17, A, page 95. As in the other graphs, the error involved in the use of the method is indicated by the closeness with which it clings to the zero line. The average negative deviation is 9.3% of the cross section values.

7. Auger borer and block methods of sampling compared.

In connection with the block method of determining the moisture content of the first ten rings and the remaining center, the auger was used to determine how closely it approximated these values. The data are shown on Graph 17, B. There are not enough determinations to make these curves significant, although it appears that they show the same general relationship to the "correct" line as the samples of the cross section shown in Graph 17, A, on the same page.

Conclusions

The determinations of the errors involved in the various methods of sampling and measurement described permit us to draw the following tentative conclusions:

1. Direct weighing of chips in the chipping method

results in an error of about 6.4%.

2. Errors in volume determinations are still unknown.

3. Increment borer methods promise to be useful. Too little work has been done to establish the correction factor.

4. The auger borer gives erratic results, which are almost always lower than the true value.